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Authenticity and Quality of Muscle Foods: Assessing Consumer Trust and Fraud Detection Approaches

Salih, Salih Mustafa

Authenticity and Quality of Muscle Foods: Assessing Consumer Trust and Fraud Detection Approaches

by

Salih Mustafa Salih

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Dedication

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Author's Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee.

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Salih Mustafa Salih

Authenticity and quality of muscle foods: Assessing consumer trust and fraud detection approaches

Abstract

Authenticity issues and fraudulent practices regarding animal products are affecting consumer confidence. Verifying the description, composition, processing or origin of foods can be challenging. To explore British and Kurdish consumers' perceptions of kebab meat products, focus groups and questionnaire surveys were applied. About 40% of participants in the UK tend to purchase fewer processed meats after the European horsemeat scandal. Issues raised by participants indicated their concerns about the declaration of species, meat content, and other ingredients incorporated in kebab and other meat products. Lack of consumer trust has been linked to authenticity issues. Reactions towards the addition of fat-replacing inulin were positive by more than half of respondents. A further study aimed to investigate the effect of commercial inulin (CI) and Jerusalem artichoke (JA) tubers as fat replacers on the eating quality and overall acceptability of kebabs. Inulin flour prepared from JA by a simple protocol presented advantages with about 10% higher cooking yield and overall acceptability when compared with CI. Levels of inulin as low as 0.5% were detected in meat products using enzymatic assay, which could be relevant to detect additives and enforce labelling requirements.

The authenticity (origin and species) was investigated in fish samples from commercial markets in Erbil, Kurdistan Region of Iraq (KRI). The declared fish species was checked using DNA barcoding with Cytochrome b region. A 10 % rate of mislabelling occurred only for wild common carp (*Cyprinus carpio*), with 9 out of 12 discovered to be the related species goldfish (*Carassius auratus*), which was deemed to be accidental rather than deliberate fraud. Such occurrences were from street markets and fishmongers, while none were from supermarkets. Wild and farmed common carp samples were not discriminated by DNA barcoding. Further fingerprinting using compositional profile and near-infrared spectroscopy (NIRS) together with chemometric analysis aimed to predict composition and discriminate between wild and farmed common carp and species identity. NIRS-predictions of composition and some macro-minerals of fish have a strong correlation with the references. NIRS with chemometric analysis is promising, but were not satisfactorily accurate for micro-minerals. Even with no clear solution from principal component analysis (PCA), NIRS-PCA may contribute to discriminating sample groups, but not for authentication when used alone. Having reliable techniques for authentication of food of animal origin may discourage deliberate replacement in retail, wholesale and international trade, and may contribute to reductions in food mislabelling, therefore protecting consumers from fraudulent practices.

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List of Abbreviations:

*a**	Redness
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
*b**	Yellowness
BLAST	Basic local alignment search tool
BOLD	Barcode of life data base
BS	British Standards
BSE	Bovine spongiform encephalopathy
CE	Capillary electrophoresis
CI	Commercial inulin
COI	Cytochrome c oxidase subunit I
CP	Control product
Cytb	Cytochrome b
DNA	Deoxyribonucleic acids
DP	Degree of polymerisation
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration (USA)
FG	Focus groups
FSA	Food Standards Agency (UK)
FSAI	The Food Safety Authority of Ireland
FSIS	Food Safety and Inspection Service (USA)
G	Gram
GC	Gas chromatography
GDA	Guideline daily amount
HPLC	High-performance liquid chromatography

Continues from previous page

ICP-OES	Inductively coupled plasma optical emission spectrometry
IEF	Isoelectric focusing
ISO	International Standards Organisation
JA	Jerusalem artichoke
KMP	Kebab meat products
KRI	Kurdistan Region of Iraq
*L**	Lightness
LACORS	Local authorities coordinators of regulatory services
LC	Liquid chromatography
LV-SEM	Low vacuum- SEM
ME	Minimum evolution
Min	Minute
MIR	Mid-infrared
Mis	Mislabelling
ML	Maximum likelihood
mM	Millimolar
Mm	Millimetres
MRM	Mechanically recovered meat
MS	Mass spectrometry
Mt	Metric tons
mtDNA	Mitochondrial DNA
µg	Microgram
µm	Micrometre
NIR	Near-infrared
NIRS	Near-infrared spectroscopy
NJ	Neighbour joining
NMR	Nuclear magnetic resonance

Continues from previous page

PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
PLS	Partial least squares
PUFAs	Polyunsaturated fatty acids
QUID	Quantitative ingredient declaration
RAPD	Random amplified polymorphic
RFLP	Restriction fragment length polymorphism
RPD	Residual predictive deviation
SACN	Scientific Advisory Committee on Nutrition (UK)
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	Scanning electron microscope
SEP	Standard error of prediction
SSR	Species specific repeat
TPA	Texture profile analysis
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
WBS	Web-based survey
WHO	World Health Organization
wt.	Weight

Chapter 1

General Introduction and Literature Review

1.1 General introduction

Food is one of the most basic needs for human life (Axelson, 1986, WHO, 2013). Animal products are among the main food items for agribusiness chains (Speedy, 2003), and an important source of many essential nutrients such as protein, fats, minerals, vitamins (Weiss *et al.*, 2010), and, in the case of fish, rich in polyunsaturated fatty acids (PUFAs) (Weichselbaum *et al.*, 2013). Data from the Food and Agriculture Organization (FAO) of the United Nations indicate a rapid growth in meat and fish productions due to the growing demand (Speedy, 2003).

The demand for information and reassurance regarding food content and its origin is on the increase among consumers (Vinci *et al.*, 2013). Ensuring the safety, quality, and authenticity of food along the supply chain is a major challenge (Aung and Chang, 2014). The process in which a food can be verified as complying with its labelling description is called authenticity (Danezis *et al.*, 2016). Generally authentication issues can be categorized into the areas where fraud is most likely to occur: meat origin, meat substitution, meat processing treatments, mislabelling and addition of non-meat ingredients (Ballin, 2010, Mohanty *et al.*, 2013).

One of the earliest forms of regulation of commercial enterprise in ancient time was the protection of the public from fraud in the marketing of food products (Hutt and Hutt, 1984). Food labelling legislations are designed to ensure that the food is properly described in order to protect the consumers from being sold an inferior product with false description, and to protect the honest traders from unfair competition, as well to maintain consumer confidence (Dennis, 1998).

However, labelling of food products alone cannot ensure the safety, quality, and authenticity of food products (Aung and Chang, 2014, Charlebois *et al.*, 2014). This is because the supply chains for many food products are long and may involve addition of ingredients at several stages of processing before they are incorporated into a food product ready for consumption (Everstine *et al.*, 2013b). This distance between food producers and consumers has increased due to the globalization of the food trade (Aung and Chang, 2014).

Therefore, the modern food supply chain network has become very extensive, complicated and enhanced due to consumer demand, which increases the possibility of food fraud (Spink *et al.*, 2010, Spink and Moyer, 2011, Everstine *et al.*, 2013b). Therefore, an inquiry into the integrity of the UK food was commenced by the UK government in the wake of the horsemeat incident, and one of the suggestions was to establish a national food crime unit, to protect consumers from food fraud incidents in the future (Elliott, 2014).

Recent incidents of food fraud in food of animals include the European Union (EU) horsemeat scandal (Elliott, 2014), the case of halal products containing pork (Nakyinsige *et al.*, 2012), traces of donkey DNA (Deoxyribonucleic acids) in processed meat products in South Africa (Cawthorn *et al.*, 2013), and the high incidence of seafood fraud in the United States (Warner *et al.*, 2013). These incidents, in particular horsemeat, have made the international headlines and attained media attention (European Commission, 2013). As a result, important questions were raised regarding traceability, food safety, consumer protection and how authorities can ensure that the food we eat is indeed what it is labelled to be (Stamatis *et al.*, 2015).

The growing number of food fraud cases can be partially attributed to the increase in world trade and emerging new markets, as well as the steady increase of food prices all around the world (Charlebois *et al.*, 2016). Furthermore, the continued occurrence of food scandals, mostly for economic reasons, has contributed to increased consumer interest in the food they eat and how it is produced (Carvalho *et al.*, 2015a).

On the other hand, processed meat products are commonly high in fat (Biesalski, 2005, Weiss *et al.*, 2010), and the link between excessive consumption of meat products rich in fats and some chronic diseases was highlighted in several epidemiology studies (Käferstein and Clugston, 1995). Consumers believe that food should contribute directly to their health, and not only should it satisfy hunger and provide necessary nutrients, but it should also prevent nutrition related diseases (Roberfroid, 2000, Menrad, 2003). This can be achieved with modification of normal components by the substitution of detrimental components with functional ingredients that are considered beneficial for health (Fernandez‐Gines *et al.*, 2005).

Several functional ingredients have been used for these purposes in the food industry. For instance, soy protein has been widely used in meat, poultry and seafood products (Waggle *et al.*, 1981), and commercial inulin from chicory in several meat products (Mendoza *et al.*, 2001, Selgas *et al.*, 2005, Menegas, 2013, Keenan *et al.*, 2014, Mehta *et al.*, 2015).

However, the challenge for both the scientific community and the food industries is to give consumers the assurance that these new food products are not a new opportunity for profits that may mislead consumers, but a genuine attempt toward better and healthier food (Roberfroid, 1999). Some of those ingredients

used as functional food supplements, such as soy protein, can be subject to fraudulent substitution with cheaper animal protein (Macedo-Silva *et al.*, 2001, Koppelman *et al.*, 2004). Furthermore, inulin is also becoming a target for substitution with low-priced sweetening products, such as glucan, which have a similar profile to inulin (Wang *et al.*, 2010).

As a consequence of the continuing occurrence of food scandals and incidents, consumers are highly demanding of high quality food with integrity, safety guarantees and transparency (Beulens *et al.*, 2005, Regattieri *et al.*, 2007, Trienekens and Zuurbier, 2008), as well as having accurate and reliable labelling information (Primrose *et al.*, 2010).

To sum up, it is clear that authenticity issues affect consumers' confidence and trust. Therefore, in order to build consumers' confidence, it is essential to have a traceability system in place, following regulations and standards (Charlebois *et al.*, 2014). The availability of analytical tools plays a fundamental role in ensuring the authenticity of food in our modern society (Dennis, 1998), and is therefore essential in monitoring food quality and authenticity (Shieber, 2008). These analytical methods should be robust, efficient, sensitive, and cost effective in order to guarantee the safety, quality, and authenticity of foods in compliance with legislation and consumers' demands (Cifuentes, 2012). Therefore, assessing consumer trust and investigating some fraud detection approaches would be very important for solving the gaps in the current knowledge of this research area.

1.2 Literature review

The purpose of the current literature review was to give an overview of the existing research work on consumers' perception and the potential issues of food authenticity, as well as the fraud detection approaches used in food from animals.

First, a general background on the production, consumption and perception of food from animal products is given, followed by an overview of the relevant labelling regulations and requirements. The review also presents a historical perspective on food authentication and the potential issues with fraud in general, drawing on some previous and recent food fraud incidents. Finally, a range of analytical methodologies that are currently available for authentication of food from animal products is discussed.

1.2.1 Global production and consumption of animal products

Meat and fish products are considered major sources of protein (Biesalski, 2005). The production of foods from animal sources is in increasing due to growing demand (Speedy, 2003). For example, global demand and consumption of fresh fish has increased (Vannuccini, 2004, FAO, 2009), and there has been significant growth in the fresh fish market in the UK in particular during recent years (Nga, 2010).

The estimation of fish contribution of animal protein intake per capita is about 15% of all sources of protein (Béné *et al.*, 2015). The consumption of fish is considered a healthy source of protein, and is rich in PUFAs, which are associated with several health benefits (Weichselbaum *et al.*, 2013).

The global production and consumption of meat is expected to continue to rise, from 233 million metric tons (Mt) in 2000 to 300 million Mt in 2020. However, the pattern of consumption is varied; the annual consumption of meat in the U.S. is 124kg per capita compared to the global average of 38kg (Speedy, 2003). This variation is mostly due to cultural and religious factors (Allievi *et al.*, 2015). Increasing meat consumption, especially in the western world (Rohrmann *et al.*, 2013), is due to many factors such as wealth, volume of livestock production and socio-economic status of the consumers. The consumption pattern also varies by gender, with men more likely to eat meat than women in European countries (Figure 1.1).

The UK has one of the lowest daily meat intakes, in particular of red meat, compared to other European countries (McAfee *et al.*, 2010). This was also supported by Kearney (2010), and it could be due to a number of related health scares, such as bovine spongiform encephalopathy (BSE), commonly known as the 'mad cow disease' crisis in the UK. Moreover, many researchers reported the link between excessive meat consumption and the health risk related to obesity and cardiovascular disease (McAfee *et al.*, 2010).

The negative image of frequently consuming meat is due to high fat content (Biesalski, 2005), especially processed meat products from takeaway or fast food outlets (Jaworowska *et al.*, 2013). Meat products such as sausages, burgers, pork pies and kebabs account for almost half of all meat consumed in developed countries (Kearney, 2010). Low meat intake, in particular red meat, is recommended to avoid harmful health effects (Biesalski, 2005).

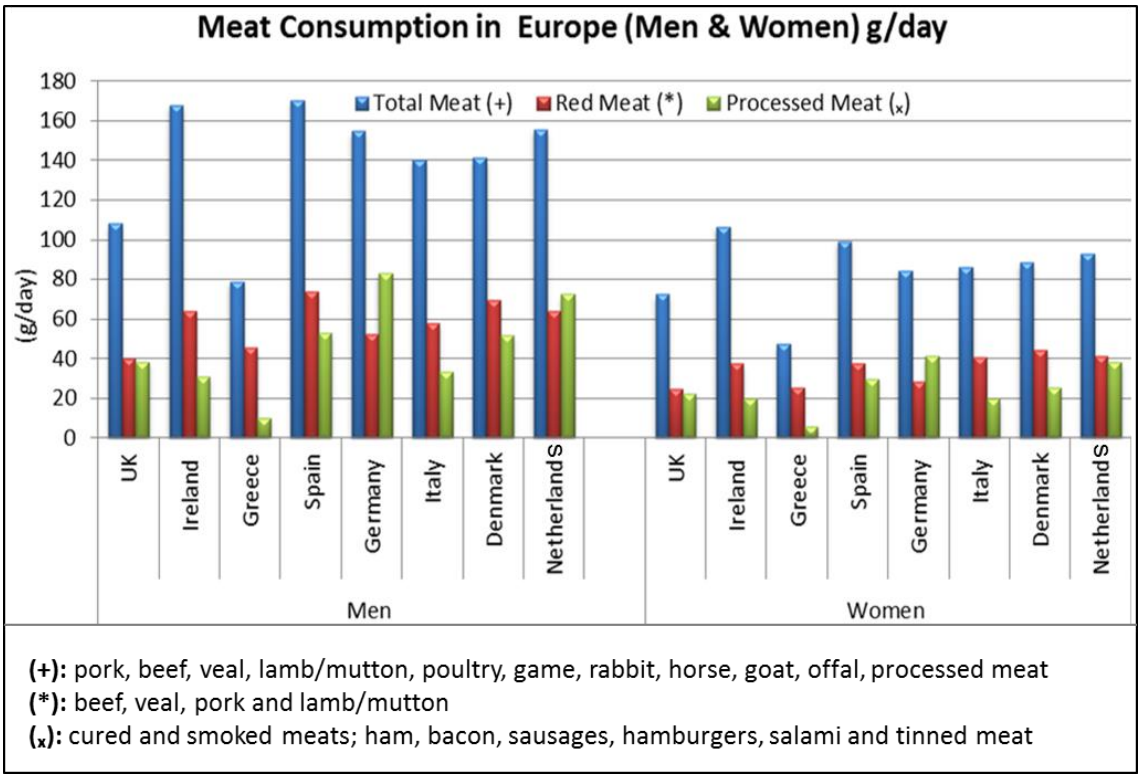


Figure 1.1: Daily intakes of meat consumption in the Europe, adapted from McAfee *et al.* (2010)

Due to the high value of meat, there is always an opportunity to substitute it with material lower in price (Alamprese *et al.*, 2013). Many consumers are concerned that the food product labelling may not reflect the content and that fraudulent practice misleads consumers (Barnett *et al.*, 2016). The choice of one product over another can reflect aspects of lifestyle (e.g. vegetarianism and organic food), religion (e.g. absence of pork from some diets), diet and health concerns (e.g. absence of allergens). Therefore, clear and correct labelling is important to inform consumer choice and support fair-trade (Ballin, 2010).

1.2.2 Qualitative and quantitative approaches to understand consumers' perceptions of food authenticity

Several qualitative and quantitative tools are available to assess consumers' perceptions depending on the research questions. Generally, focus groups (FG) as a qualitative method are frequently used in exploratory studies (Creswell, 2007) as a reliable method to gain information regarding consumer attitudes, perceptions, behaviours, habits, and experiences, rather than statistically secured facts (Morgan, 1997, Langford and McDonagh, 2003). FG roughly consist of between 6 and 12 participants per group meeting for a considerable length of time run by a trained moderator on a selected topic in a free open environment (Creswell, 2007, Onwuegbuzie *et al.*, 2009).

FG sessions can be continued until "saturation" is reached, and no new significant themes have appeared (Sim, 1998). Qualitative research is the only method that can provide rich insight into how individuals think about and understand issues in their own terms (Lytle *et al.*, 1997). Therefore, this method generates words, rather than numbers, as data for analysis compared to quantitative research (Bryman, 2008). It can be used as an alternative research method or as a supplementary approach that adds value to other research methods such as individual interviewing, participant observation, surveys and experiments (Puchta and Potter, 2004).

Surveys, on the other hand, are designed to collect information from respondents as a series of related questions to be answered by respondents from a sample of well-defined population (Czaja and Blair, 2005). There are several techniques for conducting surveys, including WBS (web-based survey), face-to-face interview, phone interview, email questionnaire, short message

service-based surveys, self-reported paper questionnaire and video conferencing (Kenett and Salini, 2011). However, WBS as a new and popular channel for gathering information is widely used in the present time and has advantages over other methods regarding the large sample size, low cost, speed of data collection, better geographical distribution and control of question order and design (Schleyer and Forrest, 2000, Cobanoglu *et al.*, 2001, Roztocki, 2001, Czaja and Blair, 2005, Kenett and Salini, 2011). The main differences between qualitative and quantitative research are summarised in Table 1.1.

Table 1.1: Difference between qualitative and quantitative research (Islam, 2005)

Aspects	**Qualitative research**	**Quantitative research**
Aim	Exploration of participants' meaning. Understanding, generation of theory from data	Search for casual explanations. Testing hypothesis, prediction, control
Approach	Broad focus, process oriented, Context-bound, mostly natural setting. Getting close to the data	Narrow focus, product-oriented, context-free, often in artificial setting
Sample	Participants, informants, sampling units such as place, time and concepts. Flexible sampling which develops during research	Respondents, subjects. Sample frame fixed before research starts
Data collection	In-depth non-standardised interviews. Participant observation/ fieldwork. Documents, photographs, videos	Questionnaire, standardised interviews. Tightly structured observations. Documents, randomized controlled trials
Analysis	Thematic, latent content analysis such as grounded theory, ethnographic analysis	Statistical analysis
Outcome	A story, an ethnography, a theory	Measurable results
Relationship	Direct involvement of researcher. Research relationship close	Limited involvement of researcher, research relationships distant
Validity	Trustworthiness, authenticity	Internal/ external validity, reliability

Generally, consumers' acceptance of goods or services is the degree of satisfaction measured quantitatively or qualitatively (Kenett and Salini, 2011). FG as a qualitative method are often used in market research to focus on different social groups for special products or services, and can be a bridging tool to eliminate the social gap between markets and consumer perceptions (Puchta and Potter, 2004), in contrast questionnaire surveys are used to measure customer satisfaction based on the gap between customer expectations and marketing (Kenett and Salini, 2011). Often the focus group discussions are used as the first stage of investigation, followed by quantitative research such as questionnaire surveys (Creswell, 2007).

There are many factors that may influence or determine consumers' preferences, such as cultural factors, which mostly result from environmental conditions such as climate, technology, geography and food availability; social factors, including the influence of friends, relatives and family members; personal factors, such as age, education, and psychological characteristics; and situational factors, such as income and employment status (Axelson, 1986).

Many studies have used a combination of qualitative and quantitative methods to explore consumer attitudes toward a particular aspect of a food product (Verbeke and Brunso, 2005, Verbeke *et al.*, 2007, Lee *et al.*, 2012, Claret *et al.*, 2014). A strong correlation between reading the label and gender was observed in a survey conducted in Austria 2015 for measuring consumer perception towards mislabelling and to use a device to self-authenticate. It was found that female respondents are more likely to look at labels and country of origin than men. Moreover, well educated people have less trust toward regulators and industry to monitor labels and take action against food fraud. Other

sociodemographic factors such as age, sectors of employment, and frequency of visits at grocery stores were considered insignificant (Charlebois *et al.*, 2016).

An example of fraudulent mislabelling is shown in a survey conducted in the UK by the Food Standards Agency (FSA) in 2007 that investigated the authentication of fish described as “wild”. Results showed that 10% of 128 wild sea bass analysed were mislabelled and found to be farmed sea bass, while this was 11% for sea bream and 15% for salmon (FSA, 2007). In addition, in a survey published by Oceana (an international organization for protecting the world’s oceans) on seafood fraud within the U.S., over one-third of the collected samples were mislabelled (Warner *et al.*, 2013).

On global scale, seafood products were among the top foods that were mislabelled in about 10% of all fraudulent foods (Moore *et al.*, 2012). Another public survey conducted by the UK FSA revealed that labelling of food products with their country of origin is highly demanded by consumers (FSA, 2007).

Looking at the impact of the recent horsemeat scandal in 2013, a key priority for all agencies involved in the food supply chain network, especially in processed meat products, is to build and restore consumers’ confidence (Barnett *et al.*, 2016).

1.2.3 Food labelling regulations for authentication of animal products

Originally, food was regulated by religious laws that enforced food standards (Hart, 1952). As long as food has been traded and the trade globalization has increased, there has been a risk of fraud (Anklam and Battaglia, 2001, Li, 2013), and this concern dates back to Greek and Roman ancient times (Sumar and

Ismail, 1995, Anklam and Battaglia, 2001, Shears, 2010). Generally, there is a requirement for the food markets at the national and international levels to label products with information regarding their composition and quantity. Proper labelling of meat content and meat species authenticity is important to ensure fair trading among producers and to enable consumers to make informed choices (Przyrembel, 2004).

Initially, the prevention of food or drink adulteration act was introduced in the UK in 1860, and was revised by the adulteration of food and drug act in 1874 (Shears, 2010, Manning and Soon, 2014). The later 1984 Meat and Meat Products legislation specifically prohibits the adulteration of meat with meat from other species (Al-Jowder *et al.*, 1997). For example, it is generally recognised as a criminal offence under sections 14 and 15 of the Food Safety Act 1990 in the UK when a food is sold that is not of the nature, substance or quality demanded by the consumer, or when it falsely or misleadingly described (Walker *et al.*, 2013).

There is a general requirement for quantitative ingredient declaration (QUID) in meat products including pre-packed and loose meat products. However, there is no requirement of QUID for loose meat products sold at catering establishments such as restaurant, canteens, clubs, public houses, school, and hospitals (FSA, 2003). There is no requirement for doner kebab sellers to provide details of the meat content when the product is sold unpacked to the final consumer. In addition, in the catering services there are no clear details of the meat species and the quantities of each meat species. Therefore, it has been suggested by LACORS (Local authorities coordinators of regulatory services), as one of the

UK local government organisations, to clarify the term doner kebab with information on the meat species contained (LACORS, 2009).

European legislation (EC Regulation 178/2002) lays down the principle and requirements of food laws, and provides the legal basis for the establishment of the European Food Safety Authority (EFSA). The protection of consumers' interests was addressed in Article 8 of the EU in order to provide the basis for consumers to make informed choices in relation to the foods they consume. It also aimed at the prevention of fraudulent or deceptive practices, the adulteration of food, and any other practices which may mislead the consumer. According to Article 17, food business operators have a responsibility to ensure and verify that food complies with all relevant food law, including rules on food labelling (European Commission, 2002).

Since early 2002, it has been a legal requirement in the EU that the seafood sector must provide the consumer with clear labelling information, including fish species name, production method of fish (wild or farmed), and geographical origin (Mohanty *et al.*, 2013). The Council Regulation No. 510/2006 highlighted restrictions on the protection of geographical indication and designation of origin for agriculture products. Its aim is to protect two types of product labelling; Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) (Montowska and Pospiech, 2012). The European Directive 2003/89 (EU, 2003) requires indication of the presence of specific allergens in meat products, whether they are used as an ingredient or in the preparation of an ingredient (Fogden, 2011).

The addition of foreign ingredients into meat products is regulated under the European Regulation 2008/1333 (EU, 2008), including any substances not normally consumed as a food but that are used for technological purposes in meat processing, preparation, treatment, packaging, transport and storage, which must be declared in lists of ingredients. For example, inulin as dietary fibre is legally classified as food or food ingredients, not as additives in European countries (Barclay *et al.*, 2010), as well as Generally Recognized as Safe status in the U.S. (Nair *et al.*, 2010). The authorities in Australia, Canada and Japan came also to the same conclusions (Franck, 2002).

1.2.4 Integrity and traceability of animal products

Food chain integrity is multi-disciplinary, covering all aspects of the food chain from producers to consumers (Hoorfar and Prugger, 2011). The term integrity typically refers to being whole or entire. The description of the product should meet the agreed specification that has been laid down in terms of expressing the total completeness of the item that is undiminished without removal of part (Manning and Soon, 2014). Food chain integrity aims to cover the aspects of microbial and chemical food safety, fraud and quality, as well as authenticity of origin (Hoorfar and Prugger, 2011).

Traceability has been defined by the European Commission in Article 18 as the: “ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution” (European Commission,

2002). Its aim is to maintain authentication of animal products through various steps within the supply chain, from the farm to the retailer (McKean, 2001).

Since early January 2005, traceability systems have become mandatory in the European Union according to the EU directive 178/2002, as useful tools to improve food safety and prevent fraud (European Commission, 2002, Folinas *et al.*, 2006). From the consumer prospective, traceability can help to build trust and increases confidence in the food system as an important tool to improve food safety (Opara, 2003, Martinez and Epelbaum, 2011). Generally, having a traceability system in place allows food businesses to target food products affected by food safety issues, minimizing disruption to trade and any potential public health risk (Carvalho *et al.*, 2015a). Furthermore, in order to assure food traceability from farm to fork, the implementation of authenticity and diagnostic tests is essential to detect and prevent food safety hazards (Folinas *et al.*, 2006).

1.2.5 Authenticity of foods of animal origin

Quality control is an important aspect in the food chain. Authentication and adulteration of products are other aspects of quality control that have become more important for manufacturers, due to the increasing competitiveness in the food industry. It is essential that the product conforms to the descriptions provided by the producers or processors and that no adulterations were added (Downey, 1996, Downey, 1998).

The word “authentic” is commonly defined as something “reliable, trustworthy, of undisputed origin, genuine” (Lüthy, 1999). Consumers are constantly seeking authentic brands and authentic experiences (Fine, 2003). The use of

authenticity has been a well-known technique by marketers to boost sales. It is often used in brand management that easily helps to create unique brand identity (Aaker, 1996). Food authenticity has become a significant field of food forensics which can ensure food quality and safety to the consumers and compliance with relevant regulations (Ashurst, 1996). It is one of the most important aspects in food quality and safety (Gallardo *et al.*, 2013). In this regard foods from animal origin including meat, meat products, fish and seafood are the most susceptible products for adulteration issues due to their high values (Rodríguez-Ramírez *et al.*, 2011).

In response to the food fraud incidents, especially the horsemeat scandal, the UK government commissioned an independent review into the integrity and assurance of the food supply networking known as the Elliott Review. The review aimed to address the issues of food security and its potential impact on areas of traceability and authenticity. It also aimed to increase consumer confidence and called for a new culture and adoption of a zero tolerance approach as a core principle to food crime. It was recommended that food industry should take actions and focus on the areas where margins are tight and the potential for fraud is high (Elliott, 2014).

1.2.6 Potential issues with authenticity of foods of animal origin

Foods are often subjected to a number of undesirable processes, such as contamination, poisoning, adulteration, fraud and degradation. Whether these processes were caused intentionally and/ or accidentally, the result is that this may turn food into very undesirable or dangerous products that should be

avoided (Savov and Kouzmanov, 2009, Porcari *et al.*, 2016). However, mislabelling attributed to fraudulent substitution is most likely intentional and economically motivated for economic profit (Everstine *et al.*, 2013b).

The risks of these processes from food fraud are getting much attention from industry, government, and standards-setting organizations. This fraud is mostly conducted by food producers, manufacturers, processors, distributors, or retailers (Spink and Moyer, 2011, Moore *et al.*, 2012, Cozzolino, 2015). Due to the massive urbanization trend of the twentieth century, many people do not produce their own food. In this regard, several stages are involved in the food supply chain (growers, processors, wholesalers, distributors), which consequently increases the potential of misleading, fraudulent practices, and misspresentation (Puckett, 2004).

Food adulteration is an age-old problem (Manning and Soon, 2014). Therefore, when markets began to offer fully prepared foods, opportunities for adulteration increased dramatically (Hart, 1952). Moreover, the fraud is frequently increased since the opening of international markets and global competition mainly for economic profit. Spink and Moyer (2011) defined food fraud as “a collective term used to encompass the deliberate and intentional substitution, addition, tampering, or misrepresentation of food, food ingredients, or food packaging; or false or misleading statements made about a product for, economic gain”. They also identified seven distinct types of food fraud (Table 1.2). Each type of fraud incident generates different potential levels of authenticity issues for financial gain (Spink and Moyer, 2011). Moore *et al.* (2012) conducted a research study to set up the database of food ingredient fraud and economically motivated adulteration from 1980 to 2010, based on the information from 677 references

collected from publicly available articles in scholarly journals and general media. The authors created three categories of food fraud, including replacement of less expensive food, and addition or removal of food ingredients. The analysed results showed that replacement represented about 95% of fraud from the records in the database, while addition and removal represented only 5% and 1% respectively. Although meat products sold for human consumption should be accurately labelled regarding the meat species they contain, fraudulent or unintentional mislabelling still exists that may not be visually detectable. Meat adulteration occurs not only in imported products but also at the restaurant and retail level, where the substitution is easier to conceal (Barai *et al.*, 1992).

Table 1.2: Types of food fraud incidents (Spink and Moyer, 2011)

Term	**Definition**
Adulteration	A component of the finished product is fraudulent
Tamper	Legitimate product and packaging are used in a fraudulent way
Over-run	Legitimate product is made in excess of production agreements
Theft	Legitimate product is stolen and passed off as legitimately procured
Diversion	The sale or distribution of legitimate products outside of intended markets
Simulation	Illegitimate product is designed to look like but not exactly copy the legitimate product
Counterfeit	All aspects of the fraudulent product and packaging are fully replicated

For example, the major issues of authenticity with meat and meat products is the substitution of high value raw materials with cheaper materials such as less costly cuts, mechanically recovered meat (MRM), offal, blood, water, eggs, gluten or other protein of animal or vegetable origin (Al-Jowder *et al.*, 1997). The possible reason for the high contamination rate in processed meat products

is the accidental contamination resulting from improper handling or processing (Ballin, 2010). While in the seafood industry, several studies have revealed deliberate mislabelling, where valuable fish are substituted with those of lower value, in a greater range of species, markets and countries (Wong and Hanner, 2008, Carvalho *et al.*, 2011, Hanner *et al.*, 2011, Cawthorn *et al.*, 2012, Galal-Khallaf *et al.*, 2014, Carvalho *et al.*, 2015b, Stamatis *et al.*, 2015).

For this reason, authentication of fish and seafood products has become a crucial issue to protect consumers from fraudulent and deceptive practices (Rasmussen and Morrissey, 2008). On the other hand, Muslim consumers on a global scale are concerned about several factors concerning meat and meat products such as substituted pork, undeclared blood plasma, and use of prohibited ingredients, pork intestine casings and non-halal approved methods of slaughter (Nakyinsige *et al.*, 2012).

Verifying the authentication of halal food with analytical methods is important to guarantee reliable halal food for consumers and to build confidence and trust for buyers at the domestic market and in importing countries (Van der Spiegel *et al.*, 2012). Therefore, tracing and testing the authenticity of meat species is becoming an essential field of study not only for economic, health, religious and ethical reasons, but also to comply with regulations and ensure fair trading (Cawthorn *et al.*, 2013). Figure 1.2 shows the most susceptible areas of fraud in the meat industry (Ballin, 2010). Although most of these issues were highlighted in the below sections, some of the issues raised research questions that further analysed in particular species identification, origin authentication of production method, and the additions of non-meat components such as additives.

Another two authenticity issues were also included in Figure 1.2; genetically modified (GM) food and Halal/kosher are new categories. The main factors leading to food fraud has been highlighted as follows (O'Mahony, 2013):

1- The financial crisis
2- Rising food prices
3- Demand for cheap food
4- The complex of food supply chain
5- Pressure on control services
6- Low risk of detection
7- Lack of focus on detecting fraud
8- Lack of a strong deterrent (penalties)

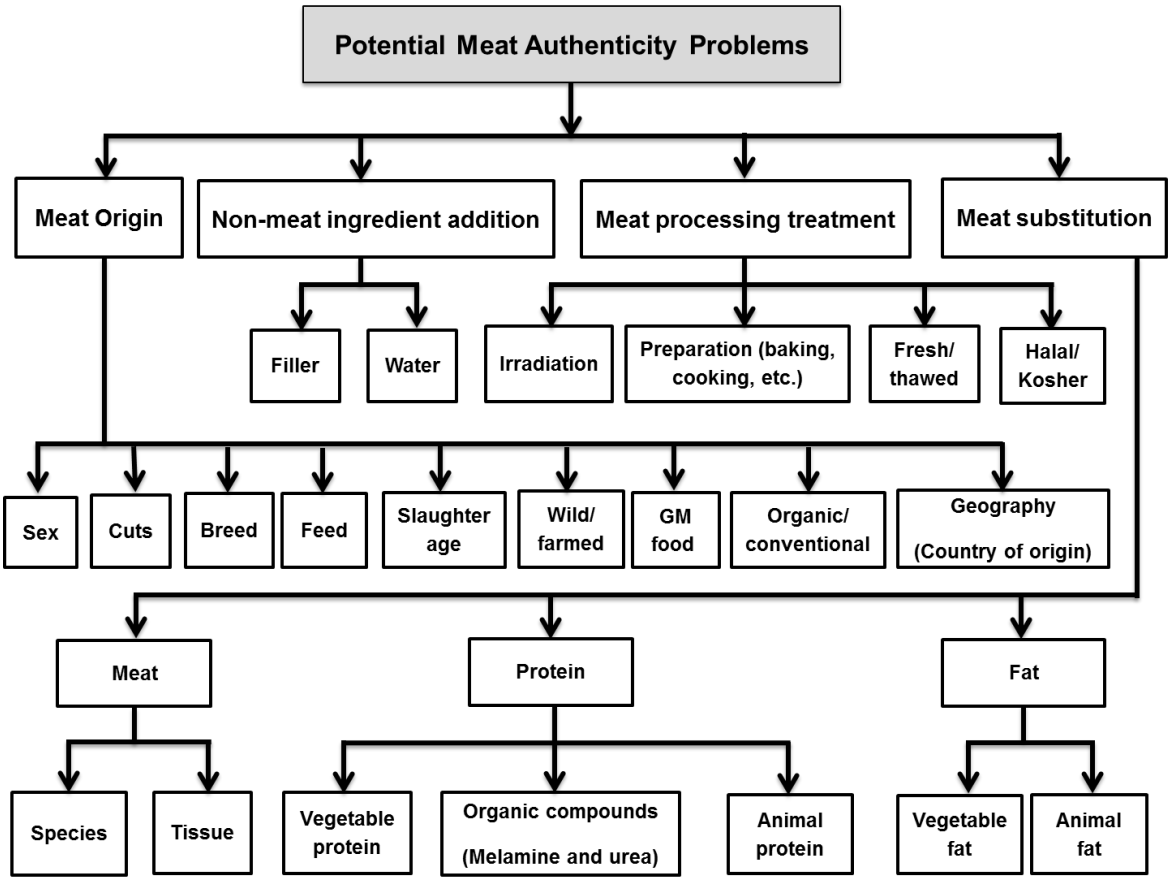


Figure 1.2: Potential meat authenticity problems, adapted from Ballin (2010)

1.2.6.1 Meat substitution

Meat products are often targeted for species substitution and adulteration due to their high market value (Cawthorn *et al.*, 2013). Meat and meat products are often subjected to adulteration with lower value meat which is often undeclared in product labelling. Moreover, the substitution or adulteration of meat occurs due to the following occasions: substituting meat from one species to another, adding poorer quality meat such as MRM, and using cheaper proteins such as from vegetable origin (Lees and Popping, 2003).

On the other hand, processed meat products such as sausages and hamburgers are generally made of ground meat mixed with fat. Because minced meat is usually handled in bulk and its composition can be extremely variable and easily manipulated, it can therefore be vulnerable for adulteration and contamination (Cozzolino *et al.*, 2002a).

Several cases of meat species substitution were reported, including the detection of 68% of the collected processed meat products containing species which were not declared on the product labelling in South Africa (Cawthorn *et al.*, 2013). Undeclared horse meat was identified in 10 of 27 (37%) burger samples by the Food Safety Authority of Ireland (FSAI) in trace levels except for one which had 29.1% (O'Mahony, 2013).

1.2.6.2 Meat origin

The potential issues with the identification of meat origin can be categorised as follows (Figure 1.2); sex, meat cut, breed, feed intake, slaughter age, wild versus farm, organic versus conventional, and geographical origin (Ballin, 2010). Fraud within the same species can involve at least two forms; gender and origin.

For example, beef from male animals is commercially more valuable, tender and better quality than beef from female. Therefore, dishonest sellers often sell the beef from females as from male animals (Shears, 2010).

Several methods have been used to determine the animal sex; however, the focus seems to have shifted from traditional PCR towards real time PCR (Parati *et al.*, 2006). For differentiation between different meat cuts such as chuck, brisket, sirloin, and shank, visual inspection can be used, or chemical constituents can be used to differentiate including the amount of collagen (Ballin, 2010). Moreover, identifying the breeding origin is essential to certify animal origin and quality of animal products (Sasazaki *et al.*, 2007, Dalvit *et al.*, 2008).

Slaughter age is another potential problem in food authenticity, and is important as the meat from young animals is often more valuable than from older animals; for instance, the highest priced veal compared to beef and lamb compared to mutton (Ballin, 2010).

1.2.6.2.1 Origin of production methods

Informing consumers about the production method (i.e. wild or farmed) is important. Namely, farmed and wild specimens carry different hazards and are subjected to different regulations and analytical control. In other words, commercially farmed seafood may contain residues of veterinary drugs whose presence is unlikely in wild. In contrast, wild specimens may contain parasites that are harmful to humans, while rarely seen in farmed seafood (Martinez *et al.*, 2009). There is a high price difference between farmed and caught fish. For instance, farmed salmon costs about £5/kg while line- caught cost around £15/kg. Therefore, the FSA conducted a snapshot survey into the labelling of

fish for sale. They found that 15% of retailers provided consumers with no information, or incorrect information about the fish identity or their origin. This misleading was higher with small businesses such as fishmongers (Shears, 2010).

The majority of wild fish species have premium prices over farmed fish (Bell *et al.*, 2007, Morrison *et al.*, 2007, Arechavala‐Lopez *et al.*, 2013), because farmed fish have different environments, stocking densities and feeding system (Arechavala‐Lopez *et al.*, 2013). However, the rising concern among fish consumers is whether the nutritional value of farmed and wild fish is equivalent, particularly in terms of lipid composition (Cahu *et al.*, 2004). The main restrictions in the production of organic meat are in the use of veterinary drugs (European Commission, 2008), and use of synthetic fertilisers and pesticides is restricted according to the EC Regulation 2092/91 (Angood *et al.*, 2008). Therefore, verifying the organic products is important. For example, all organic products sold in the UK must be certificated with government standard, and about 70% of the organic products are certified with the Soil Association's organic symbol (Shears, 2010).

1.2.6.2.2 Geographical origin

In recent years there is an increasing demand by consumers for high quality food products with reliable information on geographical origin (Luykx and Van Ruth, 2008, Montowska and Pospiech, 2012). Geographical origin, in particular country of origin, is often required for certain foodstuffs (Dennis, 1998).

In the last few years, several studies were conducted, aiming to determine the geographic origin of meat (Piasentier *et al.*, 2003, Boner and Förstel, 2004, Renou *et al.*, 2004, Shintu *et al.*, 2007). This increasing interest and the consumer awareness of claimed country of origin could be due to one of the following reasons, or a combination, including sensory qualities associated with regional products, health, patriotism, media attention, less trust and confidence in the quality and safety of import products, concern about animal welfare and environmentally friendly production methods, food scares (e.g., BSE, Food and Mouth), and malpractices of some international food producers (Kelly, 2003).

The EU passed appropriate legislation to recognize and support the potential of differentiating quality of products based on regional origin (Dimara and Skuras, 2003, Montowska and Pospiech, 2012).

1.2.6.3 Processing treatments

Generally, freezing and thawing of meat affects the quality (Thyholt and Isaksson, 1997). Proper labelling of whether meat products are fresh or thawed is important to ensure fair-trading and enable consumers to make informed choices. However, selling thawed products as fresh is commonly practiced as a target of adulteration (Ballin and Lametsch, 2008). This is due to the high price and quality attributes of fresh compared to thawed products (Šimoniová *et al.*, 2013).

To differentiate between fresh and thawed meat, several analytical methods are available, including sensory evaluation, Bio imaging, DNA based techniques (Ballin and Lametsch, 2008, Ballin, 2010), enzymatic assays (Chen *et al.*, 1988, Toldrá *et al.*, 1991, Sen and Sharma, 2005, Šimoniová *et al.*, 2013), and

spectroscopy (Guiheneuf *et al.*, 1997, Thyholt and Isaksson, 1997). Another processing treatment is the irradiation of foods, particularly MRM which is most frequently subjected to irradiation (Marchioni *et al.*, 2005).

1.2.6.4 Non-meat ingredients addition (dietary fibre-inulin) - case study

Different food additives are used in food industry for different purposes, such as binding agents, colorants, aromas, preservatives (Ballin, 2010), fat substitution and/or emulsifiers (Mendoza *et al.*, 2001). Water could also be added into meat products to sell it for the price of meat as a fraudulent practice (Ballin, 2010). However, due to the increasing demand by consumers and the global competition of meat products, meat industries are required to produce healthier meat products. These demands cause the meat industry to establish new processing technology and involve new ingredients that improve quality of the meat products (Weiss *et al.*, 2010).

Generally, dietary fibres are incorporated into meat products as a fat substitution as one of the most dynamically developing branches of the production of low-calorie foodstuffs. This is due to its water retention property, improved cooking yield and neutral flavour. In addition, it has health benefits and increases the bulk of the product (Biswas *et al.*, 2011). However, some of these dietary fibres, such soy protein, can be subjected to fraudulent substitution as replacement for animal protein (Macedo-Silva *et al.*, 2001, Koppelman *et al.*, 2004).

Dietary fibres are a part to the broad category of carbohydrates that are classified into soluble and fermentable, such as inulin, and insoluble and non-fermentable, such as cellulose (Carabin and Flamm, 1999).

However, due to the higher cost of inulin compared to other sweeteners such as glucan, inulin becomes the target of adulteration for economic gain. Some inulin frauds involve the addition of preparations based on simple and complex sugars which can be altered to simulate the natural carbohydrate profile of inulin (Wang *et al.*, 2010). Chicory as a major source of inulin was also adulterated in ancient times, when chicory was substituted with roasted carrots and turnips (Shears, 2010).

The WHO recommendation for daily intakes of non-starch polysaccharides is 16-24g or 27-40g for total dietary fibre (Nair *et al.*, 2010). However, the levels of dietary fibre intakes for general public around the world are below the recommended levels (Cho, 2009). Therefore, it is suggested by the National Cancer Institute (USA) to increase daily fibre intake, but it should not exceed 35g (Biswas *et al.*, 2011).

The overconsumption of inulin could pose negative effects including signs of intolerance with intakes above 20-30 g/day. A symptom of abdominal pain seems to occur after a single dose of fructans over 20g. Therefore, proper labelling of food containing inulin will give choices to consumers in order to make an appropriate amount of daily intake (Carabin and Flamm, 1999).

1.2.7 Impact of food fraud

Food fraud is defined by the FSA as “deliberately placing food on the market, for financial gain, with the intention of deceiving the consumer” (Elliott, 2014). It is a big business and consumers are certainly being cheated. It has been estimated, for example, that the annual food sector in the UK alone is worth around £70 billion, and the level of food fraud has been estimated at around 10% in the UK (Perks, 2007, Shears, 2010), about 25-30% in India (Singh and Neelam, 2011), and 25% in the United States (Wong and Hanner, 2008). Therefore, a small percentage of fraud can be worth lot of money (Shears, 2010).

The Grocery Manufacturers Association (GMA) estimates that fraud may cost the global food industry between 10 billion and 15 billion USD per year, affecting approximately 10% of all commercially sold food products (Johnson, 2014). In addition, there has always been adverse economic consequences for purchasers (Hutt and Hutt, 1984), and decreased consumer confidence which distorts their perception (Stiles *et al.*, 2011).

According to the research conducted by Spink and Moyer (2011) for defining the public health threat of food fraud, three different types of food fraud risks were identified, including direct, indirect, and technical fraud risks. Direct food fraud risk occurs when the consumer is put at immediate or imminent risk, such as the inclusion of an acutely toxic or lethal contaminant. Indirect food fraud risk occurs when the consumer is put at risk through long-term exposure, such as the build-up of a chronically toxic contaminant in the body through the ingestion of low doses. Indirect risk also includes the omission of beneficial ingredients, such as preservatives or vitamins, and the technical food fraud risk is

nonmaterial in nature. For example, food documentation fraud occurs when product content or country-of-origin information is deliberately misrepresented.

In order to minimize and/or even eliminate food authentication problems, it is necessary to have a continuous monitoring scheme along with improved analytical methodologies and stringent regulations (Premanandh, 2013). Several actions have been determined that may restore and increase consumers' confidence, including improving food traceability (Thompson *et al.*, 2005), sourcing local ingredients, provision of clear and accurate labelling, paying more attention to personal communication and reassurance, and providing clear information on food origin (Barnett *et al.*, 2016).

The food protection concept includes food quality, food safety, food fraud, and food defence (Figure 1.3). For example, a food defence risk is a public health threat that is intentional, such as malicious tampering or terrorism, and a food quality risk is an economic threat that is unintentional; whereas a food fraud risk is economically motivated and intentional, but is not intended to be a threat to public health (Spink and Moyer, 2011).

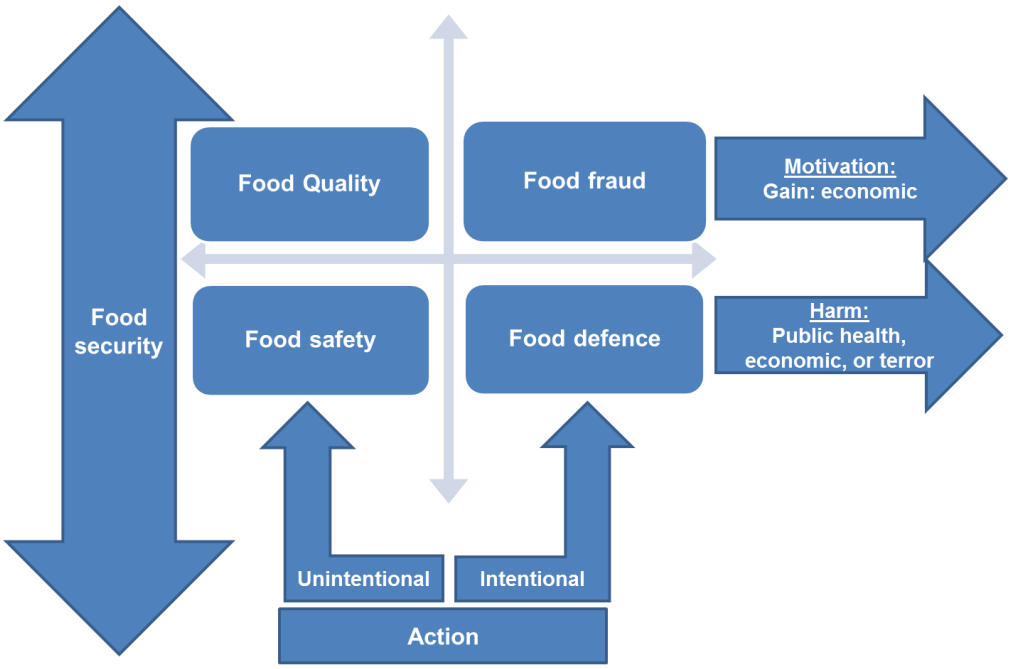


Figure 1.3: The food protection risk matrix, adapted from Spink and Moyer (2011)

1.2.8 Analytical techniques for authenticity of animal products

New technology offers many techniques to identify food authenticity issues (Wang *et al.*, 2010, Galimberti *et al.*, 2013). The 2014 Elliot Review included advice on improving laboratory testing capacity to ensure a standardised approach for testing food authenticity. It was suggested for the need of sensitive, specific, and harmonised of methods applied to detect and quantify adulteration in order to protect consumers and trade (Elliott, 2014). Therefore, to ensure and maintain consumers' confidence and satisfaction, it is essential to have reliable analytical methods to confirm food authenticity (Barnett *et al.*, 2016). Evaluation of food authentication can be performed by several analytical techniques from physical, chemical, histological, anatomical, biological and molecular analysis (Table 1.3) (Singh and Neelam, 2011, Sentandreu and Sentandreu, 2014).

Table 1.3: Examples of investigated meat and seafood products for authenticity purposes with analytical methods

Investigated Product	Country of investigation	Potential authentication problem/ analytical aim	Method of detection	References
Beef burger	United Kingdom	Beef burger contained horsemeat	Real time PCR	Nixon *et al.* (2015)
Beef	Denmark	Sex determination of beef	Real time PCR	Ballin and Madsen (2007)
Atlantic salmon	Spain	Authentication of Atlantic salmon from other close related fish	TaqMan real-time PCR	Herrero *et al.* (2011)
Beef cattle	Japan, Australia	To discriminate between Japanese and Australian beef	PCR-RFLP	Sasazaki *et al.* (2007)
Minced and canned beef meats	Iraq	Distinguish beef meats from horse and donkey meat	PCR- RFLP and PCR- SSR	Jaayid (2013)
Halal products	United Kingdom	Contained trace of pork DNA	DNA methods	FSA (2013a)
Beef breed	Italy	Breed identification in four native Italian beef breeds	Microsatellites	Dalvit *et al.* (2008)
Chicken meat	United Kingdom	Contaminating chicken meat with other meat species	Proteomic	Sentandreu *et al.* (2010)
Sausage	Mexico	Undeclared animal species	Immunodiffusion	Flores‐munguia *et al.* (2000)
Meat products	Turkey	Undeclared animal species	ELISA	Ayaz *et al.* (2006)
Hamburgers	Brazil	Undeclared soy protein	Indirect ELISA	Macedo-Silva *et al.* (2001)
European Sea Bass	United Kingdom	Differentiate wild from farmed origin	Isotopic analysis	Bell *et al.* (2007)
Gilthead Sea Bream	Scotland	Differentiate wild from farmed origin	Isotopic analysis	Morrison *et al.* (2007)
Corn-fed chicken	United Kingdom	Has chicken really been corn-fed? (Production method)	Isotopic analysis	Rhodes *et al.* (2010)
Poultry breast and dried beef	France, Germany, Hungary, Brazil, and Switzerland	Determination of geographical origin	Isotopic with element analysis	Franke *et al.* (2008)
Cooked pork meat products	Argentina	Detection of soybean and whey protein, and field bean protein	Electrophoresis (SDS-PAGE)	Lopez *et al.* (2006)
Fish species	Italy	Distinguish swordfish, blue marlin and Mediterranean spearfish	IEF	Renon *et al.* (2005)
Bovine meat	Chile	Breed identification	IR spectroscopy	Alomar *et al.* (2003)
Horse mackerel	Japan	To detect whether fish has been frozen-thawed	NIR	Uddin and Okazaki (2004)
Lamb muscle	France	To identify authentication of animal feeding	Head space GC-MS	Vasta *et al.* (2007)
Heat-processed meat products	Spain	Undeclared soy protein	HPLC	Castro *et al.* (2007)
Chicken meat	Czech Republic	Discrimination between fresh and thawed meat	Enzymatic assay	Šimoniová *et al.* (2013)
Buffalo	India	Fresh versus thawed buffalo muscle	Electron microscopy	Sen and Sharma (2004)
Lamb	United Kingdom	Identifying organic and conventionally-produced lamb	Eating quality fatty acid composition	Angood *et al.* (2008)

The initial methods of food identification were based on morphological characters such as flavour, colour, shape, taste and appearance (Winterhalter, 2006). However, molecular techniques such as PCR have become indispensable for meat authentication (Vlachos *et al.*, 2016). The availability of analytical methods is often based on DNA and protein identification methods (Wang *et al.*, 2010, Galimberti *et al.*, 2013).

However, DNA methods for determination of animal species have advantages over protein methods regarding their higher thermal stability, the presence of DNA in the majority of cells and their more informative nature. By contrast, the protein based methods are usually subjected to denaturation during heat and pressure processing which consequently makes the detection of species present in the processed sample more difficult. Accordingly, the choice of analytical technique has a significant influence on the limit of detection (Dooley *et al.*, 2004, Ballin *et al.*, 2009, Azmi *et al.*, 2011, Cammà *et al.*, 2012).

When considering specificity and sensitivity of the method for authenticating meat species, molecular methods based on DNA that require expensive laboratory equipment and certain levels of experience are needed for meat species authentication. Whereas, to save time and cost for regulatory purposes, immunological assays such as Enzyme-Linked Immunosorbent Assay (ELISA) are most frequently used in the food industry to identify meat species and non-meat ingredients such as soy protein and gluten in meat products (Asensio *et al.*, 2008). Numerous methodologies have been developed for identifying and/or distinguishing between species (Folmer *et al.*, 1994, Wolf *et al.*, 2000, Ward *et al.*, 2005, Mueller *et al.*, 2015). Other analytical techniques were used to discriminate between wild and farmed fish including microsatellite markers

(Karaiskou *et al.*, 2009, Hosseinnia *et al.*, 2014), nuclear magnetic resonance (NMR) (Mannina *et al.*, 2008), near-infrared spectroscopy (Xiccato *et al.*, 2004, Ottavian *et al.*, 2012), chemical and isotopic analysis in sea bass (Bell *et al.*, 2007).

For discrimination between farmed and wild common carp, Yeganeh *et al.* (2012) used the chemical composition and fatty acid profile. Volatile compounds were also used to discriminate between wild and farmed common carp (Mahboob *et al.*, 2009). Figure 1.4 shows an overview of some analytical techniques that are used to identify food authenticity, especially for species identity (Hsieh, 2006). Some of these analytical tools were used in this study as experimental approaches, including near-infrared spectroscopy and DNA barcoding or sequencing, and chemical composition.

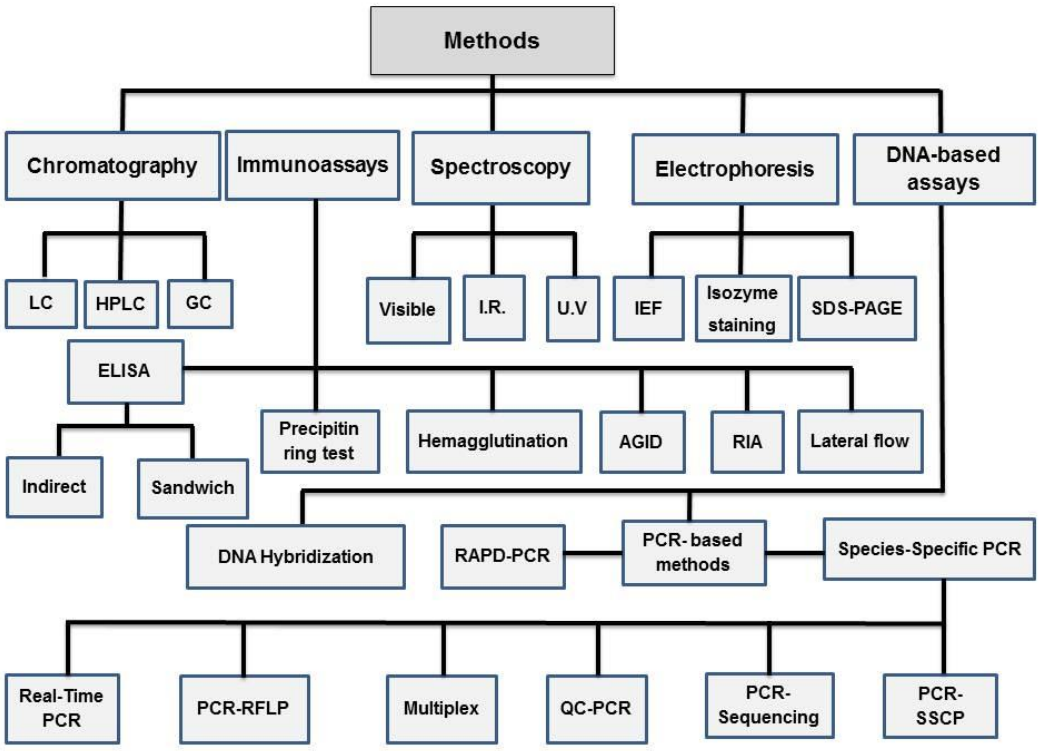


Figure 1.4: Methods for meat species identification, adapted from Hsieh (2006)

1.2.8.1 Molecular techniques

Due to the chemical stability of DNA, even in highly processed food, the detection of DNA is one of the most powerful tools for food authenticity. Therefore, the use of DNA markers as traceability tools for ingredient authenticity in food matrices has been widely investigated (Sforza, 2013). The quality and quantity of extracted DNA from food products tend to decrease with food being processed due to the physical, chemical and enzymatic treatment that can result in a noticeable decrease in DNA fragment size (Burns *et al.*, 2016).

Therefore, the selection of an appropriate analytical approach depends on many aspects, including the level of genetic variation of the analysed species, the time needed for the analysis, cost and effectiveness ratio of the technique and the expertise and availability of laboratories (Sforza, 2013). The availability of analytical methods for species identification is often based on DNA techniques (Ballin *et al.*, 2009, Wang *et al.*, 2010, Galimberti *et al.*, 2013). The comprehensive reviews by Rasmussen and Morrissey (2008), Rasmussen and Morrissey (2009), Teletchea (2009), and Hellberg and Morrissey (2011) highlighted the DNA-based methods for fish species identification.

The review of Fajardo *et al.* (2010) reported that the key point of running a successful PCR for the purpose of species detection is to choose adequate genetic markers to develop the assay. The review also shows the most commonly used markers which come from mitochondrial (mtDNA) genes, such as the cytochrome b gene. For instance, using a mtDNA sequence will increase PCR amplification sensitivity as this marker contains many copies per cell. It

also evolves much faster than nuclear markers and contains more sequence variety that could facilitate the identification of related species.

DNA barcoding or direct sequencing is one of the most used techniques for species identification due to their rapid and cost-effective features. Basically, it is achieved by comparing the sequence of a particular genomic region (highly conserved region with universal primers that amplify large number of species) found in the targeted sample with a comprehensive reference database available from GenBank and other database. However, this method is not suitable for the detection of mixed samples (Pereira *et al.*, 2008). For example, in DNA barcoding, a standard DNA fragment of approximately 650 bp (base pairs) of the mitochondrial gene cytochrome c oxidase subunit I (COI) is used to taxonomically identify a sample to the species level (Hebert *et al.*, 2003).

DNA barcoding has successfully been used for fish identification with a success rate ranging from 98% to 100% in fish species (Ward *et al.*, 2005, Ivanova *et al.*, 2007, Smith *et al.*, 2008, Carvalho *et al.*, 2015b). In the last decade, the United States Food and Drug Administration (FDA) implemented the DNA barcoding methodology to forensically identify fish products (Yancy *et al.*, 2008).

In addition, according to a survey conducted on the DNA techniques and analysed regions across the UK, Ireland, Spain, Portugal, France and Germany, DNA sequencing approaches and the cytochrome b gene were the most used authentication test and analysed region (Griffiths *et al.*, 2014).

PCR- Restriction Fragment Length Polymorphism (RFLP) is another technique that subjects amplified PCR to restriction enzymes after DNA extraction and purification. The restriction enzyme digests DNA molecules when specific DNA

sequences named “recognition sequence” are present. It separates the restriction fragments according to the length by agarose gel electrophoresis. As a result, a different length of the restriction fragment will be produced when two meat species differ in the distance between sites. Generally, enzyme recognition sites are short in length, and the shorter the recognition sequence, the larger the number of fragments generated (Pietsch and Waiblinger, 2010).

The PCR-RFLP lab-on-a-chip technology offers useful advantages for the authentication of meat species including cattle, sheep, chicken, turkey and fish. Additionally, being simple, inexpensive and especially adaptable for routine large scale intended for inspection programs are the main advantages. However, the strategy of this technique is potentially hazardous and time consuming and the results are often variable; it is also not appropriate in analysis of meats which have been contaminated by several possible species in the sample and highly degraded or mixed-species food matrices (Singh and Neelam, 2011).

The genetic identification of different species of animal for controlling food safety and forensic science is currently possible by using many molecular approaches based on DNA, such as RFLP using the mitochondrial gene 12S rRNA for identification of meat species (Wang *et al.*, 2010). For example, the traditional identification based on morphological methods is only valid for whole or slightly processed fish; therefore, molecular methods based on PCR such as RFLP-PCR are most often used to validate fish species in highly processed fish (Teletchea, 2009).

This technique has also been used as rapid and sensitive for distinguishing minced and canned beef meats from horse and donkey meat in Iraqi market

using species specific repeat (SSR) and PCR-RFLP based on mitochondrial DNA cytochrome b gene (Jaayid, 2013). Therefore, DNA based methods especially PCR-RFLP technique is the most applicable method applied currently to identify fish species (Teletchea, 2009).

In addition to the qualitative detection of species substitution or variety of adulterant ingredients, it is important to have a quantitative method in place, especially when a contamination occurred during the raw material of supply chain of manufacturing process (Primrose *et al.*, 2010). Real Time PCR has been described as the method used for quantification and it is the application used in food quality control. The method is based on the use of a fluorescent dye or a probe, which is able to give a professional result in the initial amount of target DNA that enables real time monitoring of amplification products along each cycle (Dooley *et al.*, 2004, Fajardo *et al.*, 2008).

Different types of real-time PCR are used by the scientific community such as TaqMan real-time PCR, SYBR Green, molecular beacons, and scorpions technique (Broll, 2010). However, TaqMan and SYBR Green real-time PCR are mostly used in food authentication (Ballin *et al.*, 2009).

Real-time PCR method has been used for detection and quantification of meat species in food products. However, applying the technique in meat processing with high temperature may affect the results, and it is therefore preferred to use short amplicons (López-Andreo *et al.*, 2012) of target sequence ranged from 60-80bp in length (Levin, 2008). The application of real-time PCR has been used to determine bovine sex as a reliable and accurate method (Parati *et al.*, 2006). TaqMan real-time PCR has been used for identification and quantification of

horse, donkey, and pork species in both raw and cooked meat complex (Kesmen *et al.*, 2009), and also for the authentication of Atlantic salmon from other close related fish species (Herrero *et al.*, 2011).

There are some other methods that are used for food authentication which combine genomics and proteomics feature, known as proteogeomic techniques. Examples include PCR single strand conformation polymorphisms, (PCR-SSCP), random amplified polymorphic DNA (RAPD), or the emerging field of Peptide Nucleic Acid (PNA), and DNA fingerprinting (Sforza *et al.*, 2011).

1.2.8.2 Proteomic methods

Proteomics has been a relatively recent method used in the food industry for food authentication (Gallardo *et al.*, 2013). The general principle of this technique is to use coupled peptide and protein sequence information with the analytical power of mass spectrometry (MS). It is capable of detecting and identifying peptides from protein digests containing as little as 10^{-8} mol of protein. It can be used for qualitative and quantitative detection even after cooking or heat treatment, especially with myofibrillar peptides that are resistant to heat treatments (Primrose *et al.*, 2010).

Due to some limitations of the protein and DNA based methods in food authentication, the use of proteomics techniques is a promising strategy to address the issues of food authenticity (Sentandreu and Sentandreu, 2011). These limitations in protein based methods are with closely-related species, and the lack of stability of some proteins during food processing-. They are also labour and time consuming. DNA- based methods in processed foods are

limited to disruption of cellular integrity during processing causing the release of hydrolytic enzymes. A combination of these enzymes with heat or acidic environments can negatively affect DNA integrity, reducing the length of fragments to be amplified and consequently increasing the chances of having non-specific identifications (Sentandreu and Sentandreu, 2011, Gallardo *et al.*, 2013).

A proteomic-based method has been successfully developed and applied as robust, reliable and sensitive with species-specific peptides by LC-MS/MS for detection of chicken meat within mixed meat preparations. It was possible to detect as low as 0.5% w/w contaminating chicken in pork meat with high confidence, even after cooking (Sentandreu *et al.*, 2010).

There are several reviews (Kito and Ito, 2008, Gallardo *et al.*, 2013, Mazzeo and Siciliano, 2016), and a comprehensive text (Toldrá and Nollet, 2013) published on the principles and applications of quantitative proteomics in foods authentication as summarized above.

1.2.8.3 Immunoassays techniques

Immunoassays or immunochemical techniques are based on the specific immunoreaction between a species-specific antibody and target antigen that is used to identify and quantify a target substance in a complex mixture sample, such as the added proteins in a complex food protein mixture, as well as offer a powerful tool in meat species identification (Hsieh, 2006, Kesmen and Yetim, 2012). There are two forms of ELISA techniques: indirect and sandwich ELISA tests. In the indirect type, usually two antibodies are used, one is specific for an

antigen and the other is coupled to an enzyme. In the sandwich ELISA that is most commonly used in analysing food authentication, the antigen is bound between two antibodies: capture antibody and detection antibody. The latest antibody is coupled to an enzyme that realizes a detection change in colour. ELISA test can be a qualitative or quantitative test based on the purpose of the method (Asensio *et al.*, 2008). Among the different immunological assays, ELISA technique is the most commonly used technique in food authentication purposes (Sentandreu and Sentandreu, 2014).

Immunoassays techniques have been widely used for meat authentication proposes due to their simple implementation, affordable cost, high sensitivity and the possibility of processing a high number of samples in a short time (Sentandreu and Sentandreu, 2014). However, the disadvantage of immunoassays methods is the restricted availability of antibodies free from many cross-reactions to the related organism (Hsieh, 2006, Kesmen and Yetim, 2012).

Immunoassays methods have been used to detect and differentiate the MRM and hand-deboned meat based on having different compositions of protein content especially the amount of actin and myosin where it is reduced in MRM. Moreover, ELISA technique is also used for the detection of soy protein, casein and gluten in meat products (Lees and Popping, 2003). However, the major limitations of this technique for food authentication are the inability to discriminate meat from closely related species, and possibly the interference from other ingredients (Burns *et al.*, 2016). Additional information about the use of ELISA in food authenticity can be found in the review written by Asensio *et al.* (2008).

1.2.8.4 Electrophoresis-based methods

The general principle of electrophoresis is based on the separation of soluble protein into distinctive banding patterns. However, due to the complexity of protein binding fingerprints, is often makes electrophoresis unsuitable for the analysis of meat mixture in low levels. Several techniques are available based on electrophoretic mobility, such as isoelectric focusing (IEF), sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), isozyme staining and capillary electrophoresis (CE) (Hsieh, 2006).

Electrophoresis methods have been widely used for the detection of foreign protein, mostly soybean protein, in processed meat products (Molander, 1982, Woychik *et al.*, 1987, Asensio *et al.*, 2008), as well as in meat and fish speciation (Zerifi *et al.*, 1991, Renon *et al.*, 2005).

From electrophoresis techniques, SDS-PAGE is the most widely used for the separation of soluble protein based on their molecular size. However, the lack of sensitivity and reproducibility of the assay and the difficulty of the gel interpretation are the main disadvantages that limit this technique for meat species identification. Those drawbacks were mostly affected by the amount of protein loaded on the gel, the freshness of the meat, the age and sex of the animal, the residual blood content, the degree of heat processing and the staining techniques (Hsieh, 2006).

It is possible to improve the sensitivity of electrophoretic assay in raw meat by using isozyme staining. In this method, the gel will be stained for a particular enzymatic reaction after electrophoretic separation of meat protein extracts. The staining makes the isozymes catalyse the formation of coloured compounds via

a set of coupled reactions. Often the isozyme staining will lead to several different protein bands on the gel at different positions, and those relative positions of the isozyme markers are characteristic of the species (Prasad and Mishra, 1981). IEF technique has been used to differentiate between meat species, such as beef, mutton, lamb, pork, horse and poultry, in raw meat and heated products when one species was mixed with another species at a level of above 20% (Lees and Popping, 2003).

Although the resolution is better than SDS-PAGE, the patterns of the total protein profiles are much more complicated and less consistent. Therefore, it is recommended to use IEF for identifying individual species within families or generally, but the large number of protein bands makes the interpretation of the results impossible in meat with mixed species or in uncommon meat samples. Furthermore, due to the difficulty of controlling the enzyme activity of the unknown samples, the IEF is less reliable and its application is limited to the identification of species in raw meat (Hsieh, 2006).

1.2.8.5 Chromatographic techniques

The principle of these methods is based on the separation of chemically similar components in complex food matrices. These components include peptide, lipids, carbohydrate, amino acids, fatty acids, organic acids, nucleic acids, phytochemical and other small molecule (Ibáñez *et al.*, 2013, Danezis *et al.*, 2016).

Chromatographic techniques such as high pressure liquid chromatography (HPLC), liquid chromatography (LC), and gas chromatography (GC) (Hsieh,

2006), as well as LC or GC coupled to MS have been applied for food authentication (Cuadros-Rodríguez *et al.*, 2016, Danezis *et al.*, 2016).

Detecting adulteration in food has been achieved by HPLC techniques (Forgacs and Cserhati, 2003, Nollet, 2003). The reasons for using HPLC technique over other techniques is due to the high sensitivity, very fast response and very high separation efficiencies. Addition of non-meat protein such as soy protein, caseinate and whey protein into meat products such beef, pork, chicken and turkey can be detected by HPLC (Nollet, 2003).

1.2.8.6 Stable isotope ratio and trace elemental analysis

Traditionally this method has been used to determine the geographical origin of food products, and to identify the production method of the feeding system of animals. The discrimination of this analysis basically relies on the ratio of the most common elements in living matter, including carbon ($^{13}C/^{12}C$), hydrogen ($^{2}H/^{1}H$), nitrogen ($^{15}N/^{14}N$), oxygen ($^{16}O/\ ^{18}O$), and sulphur ($^{34}S/^{32}S$), depending on the geographical origin (Montowska and Pospiech, 2012).

Each of these isotope analyses can be used individually or in combination using stable isotope ratio with mass spectrometry (Primrose *et al.*, 2010). Since the early 1970s, isotope ratio mass spectrometry has been used to detect economic fraud in food production (Kelly, 2003). Stable isotope analysis in combination with trace element measurement could be very effective for determining the geographical origin of foods, and in some cases, it can also provide information about the production methods (Primrose *et al.*, 2010).

To demonstrate the applications of this method on animal origin, Heaton *et al.* (2008) verified the geographical origin of Brazilian, British and Irish beef, while Rhodes *et al.* (2010) successfully verified the claims that chicken have been feed a diet of at least 50% maize during their rearing period.

Furthermore, isotopic fingerprinting with chemical analysis was also successfully applied to discriminate between farmed and wild fish based on different feeding regimes and on different fish species including European sea bass (Bell *et al.*, 2007), European gilthead sea bream (Morrison *et al.*, 2007), and Atlantic salmon (Thomas *et al.*, 2008).

Piasentier *et al.* (2003) also evaluated the effectiveness of the analysis of stable isotope analysis ratio (13C/12C and 15N/14N) for authentication of feeding and geographical origin of 12 lamb meats produced in pairs in six European countries. The authors concluded that multi-element stable isotope analysis may be considered promising for the reliable evaluation of lamb meat authenticity

1.2.8.7 Histology and bio imaging analysis

Histologic methods provide an accurate tool to assess qualitative parameters in particular, for detecting specific tissue components of processed meat samples (Robba *et al.*, 2003, Damez and Clerjon, 2008, Ghisleni *et al.*, 2010). Microscopic imaging has been widely used to control meat and meat product structure (Damez and Clerjon, 2008), and to detect fraudulently added water in meat products (Prayson *et al.*, 2008).

Microscopic imaging can be divided into two fields: optical microscopy and electron microscopy (Damez and Clerjon, 2008). Histology techniques based on light and electron microscopy in combination with digital image analysis have been successfully applied to assess the meat and water content of several hotdog brands in the United States to determine if the package labels are accurate in relation to the ratio of meat to water. The obtained results revealed that in most cases more than 50% of the total weight of the hotdogs was made up of water. However, according to the federal standards in the United States, hotdogs should not contain more than 30% fat, 10% water, or a combination of 40% fat and water (Prayson *et al.*, 2008).

It is well documented that freezing produces different sizes of ice crystals depending on the rate of freezing thorough the meat tissue, and this can affect the degree of microstructure. Therefore, it is possible to discriminate between fresh and thawed meat using microscopy and electron microscopy (Ballin and Lametsch, 2008).

Light microscopy in combination with image analysis has also been used to identify the presence of different animal tissues in tortellini meat-filling in four Italian commercial brands (Ghisleni *et al.*, 2006). Furthermore, the authors assessed the filling quality by examining histological sections, followed by evaluation of the percentage area of skeletal muscle by a computerised image analysis system. They concluded that this approach can be used to estimate the quality of meat and to identify small amounts of various animal tissues in processed meat products (Ghisleni *et al.*, 2010).

1.2.8.8 Metabolomics techniques

Metabolomics is the study of metabolites, their dynamics, composition, interactions and responses to interventions or to changes in their environment, cells, tissues and bio- fluids (Gibney *et al.*, 2005, Orešič, 2009). It allows the study of multiple metabolites in a cell, a tissue or an organism (Cubero-Leon *et al.*, 2014). The concept of this method is to identify and quantify as many low molecular weight compounds as possible for the authentic material to be differentiated from adulterants or substituents by the presence or absence of a particular low molecular weight. However, more research on this method is still needed (Primrose *et al.*, 2010).

In general, metabolomic studies can be classified into three categories depending on the objective of the study, including informative, where the identification and quantification of metabolites is needed to obtain sample intrinsic information; discriminative, usually achieved by the use of multivariate data analysis techniques intended to maximize classification, PCA being the most used tool; and predictive, aiming to create statistical models to predict class memberships (Cevallos-Cevallos *et al.*, 2009). However, the metabolomic applications for food authentication are mainly discriminative and predictive (Cubero-Leon *et al.*, 2014).

Metabolite analysis has been widely used by research and control laboratories to address different issues concerning the problem of meat authentication as an efficient method (Sentandreu and Sentandreu, 2014). Although metabolomics aims to create a profile of all the metabolites present in a tissue, no single analytical method has been capable of extracting and detecting all the different molecules at once. Therefore, alongside the metabolomics analytical method, it

is essential to use fingerprinting detection methods include vibrational spectroscopic techniques such as infrared (IR) and Raman techniques), nuclear magnetic resonance (NMR) spectroscopy, or a range of MS-based techniques. However, NMR and MS-based studies have gained more importance in the research published in the area of food authentication in the last five years (Cubero-Leon *et al.*, 2014).

For example, Osorio *et al.* (2012) reported the development of a non-invasive nuclear magnetic resonance-based metabolomics approach using urine samples as a tool to authenticate the cattle production system depending on the feeding system. Separation according to production system was possible as the results showed the potential use of this approach in beef authentication.

Additionally, Jung *et al.* (2010) also discriminated between the origin of meat samples from four countries (Australia, Korea, New Zealand, and the United States) using NMR-based metabolomics, suggesting that NMR-based metabolomics is an efficient method to distinguish fingerprinting difference between raw beef samples.

Additional information on the advantages, challenges and future trends of metabolomics fingerprinting approaches for the authentication of different food commodities can be found in the review (Cubero-Leon *et al.*, 2014).

1.2.8.9 Enzymatic assay for identification of fructan (inulin)

Due to the wide applications of commercial inulin from chicory in the food industry, inulin is the target of adulteration for economic gains (Wang *et al.*, 2010). Furthermore, chicory was also adulterated in ancient times with roasted carrots and turnips (Shears, 2010). Research shows that overconsumption of inulin causes sign of intolerance (Carabin and Flamm, 1999). Therefore, it is important to identify the addition of inulin in food products for labelling purposes (Zuleta and Sambucetti 2001).

Several methods have been published for the determination of fructan (exclusively inulin) in food products for labelling purposes, such as using enzymatic/ spectrophotometric method (Prosky and Hoebregs, 1999, McCleary *et al.*, 2000, Steegmans *et al.*, 2004), HPLC (Vendrell-Pascuas *et al.*, 2000, Zuleta and Sambucetti 2001), and thin layer chromatography (Simonovska, 2000). Commercially, an enzyme assay kit Fructan HK (Megazyme International, Bray, Ireland, 2013) is used with enzymatic/ spectrophotometric AOAC (Association of Official Analytical Chemists) method 999.03 (McCleary *et al.*, 2000, Muir *et al.*, 2007).

The general principles of the enzymatic assay are to extract inulin with hot water, followed by hydrolysis with inulinase enzyme, and then the determination of the released fructose and glucose. The difference between the content of each sugar with and without enzyme hydrolysis is the amount of fructan in the food sample as described in Figure 1.5 (Muir *et al.*, 2007).

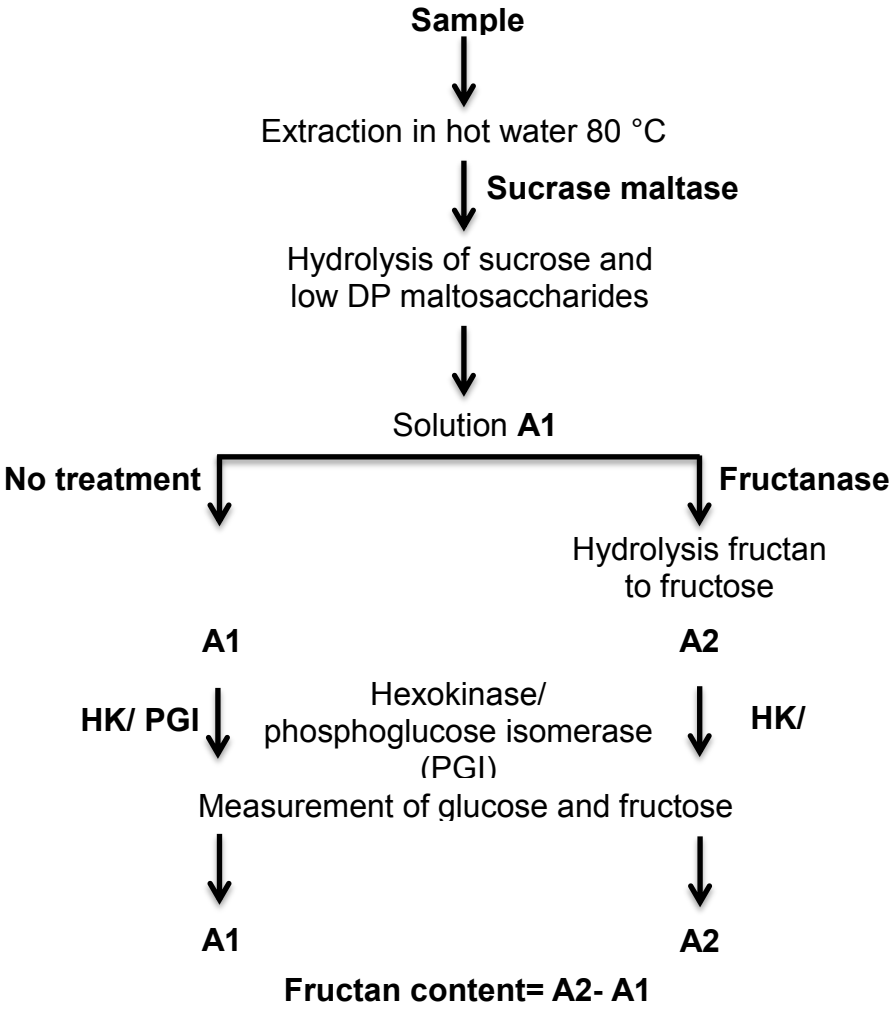


Figure 1.5: The principle of Magazine fructan enzymatic assay (Fructan HK), adapted from Muir *et al.* (2007)

1.2.8.10 Spectroscopic techniques

The study of radiation, wave propagation, absorption and the interactions between electromagnetic radiation and matter is called spectroscopy (Hildrum *et al.*, 2006). This technique usually involves three essential factors: first, a source of light; second, an element to separate the light into its component wavelengths; and finally, a detector to sense the presence of light after separation of wavelengths (Dyer and Feng, 1997).

The principle of spectroscopic techniques is based on the fact that molecules and atoms can interact with electromagnetic radiation (Cifuentes, 2012). The spectroscopic techniques or vibrational spectroscopy with a combination of multivariate statistical chemometrics techniques are widely used not only for chemical composition, but also for the authentication of origin (e.g. country, region, process, storage). The minimal sample preparation, rapidity, and ease of use in an industrial setting are the main advantages of spectroscopic methods over traditional chemical and chromatographic methods (e.g. HPLC, GC, GC-MS) for monitoring food fraud (Cozzolino, 2015).

Therefore, chemometrics in association with spectroscopy are a powerful data reduction solution used quantitatively for grouping or classifying unknown samples with similar characteristics and quantitatively for determining if samples have been adulterated (Sentandreu and Sentandreu, 2014).

Near infrared spectroscopy (NIRS) dates back to the early 1800's when Friedrich Wilhelm Herschel discovered the first non-visible region in the absorption spectrum (Davies, 2000). Near-infrared (NIR) spectroscopy method deals with the shorter NIR wavelength (800 to 2500 nm) compared to those in the mid-infrared (MIR) range (2500-1500 nm) (Manley, 2014). Initially NIR

spectroscopy was developed in animal science to evaluate the chemical composition of forages and feedstuffs (Corson *et al.*, 1999, Baeten and Dardenne, 2002, McClure, 2003). Their applications in agriculture was first used by Norris (1964) to measure moisture in grain.

Quantitative and qualitative NIRS analyses have been used successfully in many food applications (Cozzolino *et al.*, 2002b, Cozzolino and Murray, 2004, Berzaghi *et al.*, 2005, Cozzolino *et al.*, 2011). NIR spectroscopy, in combination with statistical chemometrics methods, has been widely used for authentication of several food products (Ellis *et al.*, 2012, Ottavian *et al.*, 2012, Alamprese *et al.*, 2013). NIR spectroscopy has been used as a tool for the evaluation of fish freshness based on the correlation between spectral data and storage time (Nilsen *et al.*, 2002), and also to assess the quality parameters of frozen cod in the fish industry (Bechmann and Jørgensen, 1998).

Furthermore, to verify the authentication of different food products, NIR has potential applications (Cozzolino *et al.*, 2006), including the successful discrimination between fresh and frozen fish (Uddin and Okazaki, 2004), authentication of raw and freeze-dried rainbow trout (Dalle Zotte *et al.*, 2014), and the possible distinguishment between minced chicken, pork and turkey meats as well as discriminating fresh from frozen within same meats (Al-Jowder *et al.*, 1997).

It has also been used successfully with chemometrics analysis to discriminate between wild and farmed European seabass (Ottavian *et al.*, 2012). The advantages of NIR is that it is a non-destructive technique that use less reagent (Manley *et al.*, 2008), rapid, and with simple or no sample preparation (Osborne *et al.*, 1993, Osborne, 2000, Ellis *et al.*, 2012). It allows testing of raw materials

and end products, and simultaneous measurement of major constituents in a mixed product as well as measuring several constituents or properties at the same time (Osborne, 2000).

The drawbacks of this technique are the low sensitivity of the signal, which can limit the determination of low concentration components to be determined by the use of NIR spectroscopy. It is also dependent on other chemical methods of analysis with less precision and equally empirical. The requirement of a large data set to build a robust calibration could be difficult to obtain that may eventually incorporate large variation (Osborne *et al.*, 1993, Manley *et al.*, 2008).

1.2.8.11 Chemometric methods in food authentication

Chemometrics is the chemical discipline that uses mathematics and statistics to design or select optimal experimental procedures, to provide maximum relevant chemical information by analysing chemical data, and to obtain knowledge about chemical systems (Massart *et al.*, 1988). It has a fundamental role in NIR-based calibration; methods performance in deriving calibration model is an important aspect to take into account (Centner *et al.*, 2000, Geladi, 2002).

The importance of mathematical analysis is because the NIR spectra generally consist overlapping vibrational bands that may appear non-specific and poorly resolved, and therefore chemometrics is used to sort out these spectroscopic limitations (Khodabux *et al.*, 2007). Multivariate is one of the basic methodologies in chemometrics and consists in finding mathematical relationships between a set of descriptive variables and a qualitative variable (Ballabio and Todeschini, 2009).

The development of calibration models for quantification of constituents in samples is possible by relating physical or chemical properties of the investigated samples to the absorption of radiation in the NIR wavelength range. To build the relationship modelling between the spectral data extracted from NIR and the component concentration based on the partial least square (PLS) regression or principal component analysis (PCA) as a popular linear calibration method is a great challenge (Balabin *et al.*, 2007).

Nowadays, the most favoured regression technique is the PLS regression (Leardi, 2008). The purpose of the PLS regression is to build a linear model enabling prediction of a desired characteristic (y) from a measured spectrum (x) (Nørgaard *et al.*, 2000). It was first applied to evaluate NIR spectra by (Martens and Jensen, 1982).

PCA is probably the most popular multivariate statistical technique used to characterise foodstuffs according to their origin (Monfreda, 2012). Its goal is to extract the important information from the data table and to represent it as a set of new orthogonal variables called principal components, and to display the pattern of similarity of the observations and of the variables as points in maps (Jackson, 1991, Abdi and Williams, 2010).

Validation or prediction testing refers to the calculated difference between NIR spectroscopy prediction results obtained for the constituents, properties or identification or classification, and the measurements obtained for the reference method or known identities (Næs and Isaksson, 1991). Internal validation involves validation of a calibration using the same sample set as that used for calibration development, such as in cross validation. The external validation

requires a separate, large and representative set of test objects in order to give relevant and reliable estimates of the future prediction ability of the model. This is, however, not always possible, as multivariate calibration is often done because the traditional reference method for measuring the constituent or class of interest is too expensive or slow, or is otherwise undesirable. It would be most economical to use all the data available for both calibration development and for prediction testing (Martens and Naes, 1992).

Cross-validation is a very reliable validation method where all samples are removed one at a time. After every deletion, a calibration is performed on the rest of the samples before being tested on the removed samples. The first sample is then replaced into the calibration data and the next sample removed. The procedure continues until all the samples or sample groups have been deleted once (Næs and Isaksson, 1991, Martens and Naes, 1992).

The most common statistical terms used for quantitative NIR analysis include the standard error of prediction (SEP) or standard error of cross-validation (SECV); bias; the coefficient of determination (R^2); and the ratio of standard deviation to standard error of prediction (RPD) (Osborne *et al.*, 1993, Williams, 2001).

1.2.9 Conclusion

Consumers from around the world are demanding information and reassurance as to the origin and content of their food. Determination of food authenticity is an important step in quality control and food safety. Therefore, several issues were highlighted in this review, including a drop on consumers' confidence and trust. Evidence of consumers' concerns about several food products were mentioned in this review. For example, the cases of food fraud were reported in many countries and with different food products of animal origin. Consumers also raised their concerns regarding the authenticity of food of animal products, such as species identity, the levels of meat content in meat products, the origin of meat and the addition of ingredients in meat products.

These issues may have occurred due to the lack of labelling enforcement, especially in catering establishments where there are no restrict requirements for labelling information. Other factors that may contribute to the occurrence of food authenticity issues include the financial crisis, rising food prices, demand for cheap food, the complex of food supply chains, pressure on control services, low risk of detection, lack of focus on detecting fraud, and lack of a strong deterrent (i.e. penalties). These issues could be more acute in Iraq, including the Kurdistan Region, because currently food safety is overseen by a multi-agency system. Although food laws and regulations exist in the region, these have not been updated and are not enforced to avoid authentication issues. A range of problems within food authenticity were identified from this review; some of these issues were selected as the key research questions to be investigated, as described in the next section which describes the rationale of this dissertation.

1.2.10 Rationale for the thesis

Based on the discussion provided in the literature review, it is obvious that total control of food quality, authenticity and integrity from farm to fork is not always guaranteed. Food labelling therefore may not reflect product attributes. Currently there are many questions surrounding foods of animal products (raw, semi-processed, processed, cooked) that may change consumer confidence and reduce their trust, including the origin of species identity, and food content and additives, the process history of the food, and the authenticity of the production method. Consumers require clear and accurate information on labels, not only to make informed choices about their diet and the foods they buy, but also to know how it is produced, and if it is healthy and safe. Therefore, clear labelling is essential to reassure the authenticity and integrity of foods from animals.

Little research on assessing consumers' trust and fraud detection approaches has been published on food of animal origin. The main priority areas for further research, as identified from the literature review, were the consumers' trust and the application of detection approaches. First, we set up to explore and evaluate consumers' perception and confidence, and then to assess their reactions towards additional ingredients in meat products. Then, application of reliable analytical tools to identify food authentication issues including species identity and origin of production methods were applied. The food products selected for this study included kebab meat and fish. This is due to the vulnerability of these popular items to fraudulent practices. Global increase of fish consumption and the complexity of the food chains for manufactured products may exacerbate this problem.

This thesis will investigate these aspects as detailed in below:

Chapter two investigates the consumers' attitudes toward kebab meat product authenticity using qualitative (focus group) and quantitative (questionnaire survey) approaches. A focus on the key issues and their subsequent analysis may help to restore consumer confidence and trust. A reduction in consumers' confidence on foods from animal origin, and particularly processed meat products, was recorded following the horsemeat scandal in Europe. Doner kebabs were among those products that were negatively affected. The excessive consumption of processed meat products adds to the negative public perception. Foods with high fat content and deficiency in dietary fibre are often linked to the development of some chronic diseases. Reducing the fat content negatively affects eating quality, but often sensory properties could be improved with alternative ingredients.

From ancient times, people used breadcrumbs, flour and soy in meat products and recently, commercial inulin applications are becoming an attractive possibility to substitute fat. Addition of inulin and Jerusalem artichoke (JA) into doner kebab meat would be an opportunity, firstly to assess consumers' reactions by sensory evaluation, secondly, to explore detection limits of inulin addition by the enzymatic method in prepared and other commercial products for labelling purposes, and finally, to examine the chemical, physical, and microstructure of the prepared products (Chapter 3). Fish plays a useful role in a healthy and balanced diet, and global consumption has increased over recent decades. This demand is also evident in KRI, which is not self-sufficient for producing fish. The majority of fish is imported, with only a few locally caught farmed species. However, in this region lax in labelling enforcement and

traceability, consumers are concerned about the identity of fish species, the origin of production method (wild and farmed fish), and fraudulent practices. Fraud in foods from animal origin has been widely reported, with fish labelling on the top list of occurrences, but no clear picture from KRI existed. Mislabelling occurs regardless of form and shape with whole, fillet, cooked and processed fish. Therefore, investigating the authentication and the accuracy of fish species labelling is highly timely and relevant. Molecular DNA barcoding was applied to identify fish authentication (Chapter 4).

In addition to the fish species substitutions, identifying the origin of the production method (wild or farmed) is crucial for fish authentication. The increased production of common carp has raised concerns over the quality of the farmed fish in comparison with the wild fish. Farmed fish needs different environments, stocking densities, and feeding systems, and has different prices and quality attributes compared to wild fish. Due to the difficulties of identifying the same species when they have grown in different environments, farmed fish are often sold as wild fish for financial gain. Having tools for discrimination between wild and farmed fish would minimize fraud and build consumers' confidence. NIR techniques were evaluated for potential discrimination between wild and farmed fish (Chapter 5).

1.2.11 Experimental approach

Experimental chapters include materials and methodologies specific to those studies described. Quality, traceability and authenticity of muscle food can be evaluated with a wide range of methodologies. We looked at what people think

about the authenticity issues of animal products, how important they found labelling, their reactions and acceptability towards addition of new ingredients in meat products, their perceptions since the horsemeat scandal, and the most concerning issues. We also looked on the limitations of applied techniques, how these methods helps us to understand the quality and authenticity of foods from animal, and whether we can further investigate those limitations.

In the first experiment (Chapter 2) we used two tools including FG and WBS. These tools can be used separately or together in order to assess consumers' perception of a particular aspect of a food product. In this study we used booth approaches in order to find out the difference in the obtained results using similar questions, and to make the overall approaches of this experiment robust. In the second experiment, different physicochemical, microstructure, and instrumental textures were evaluated for the eating quality and sensory attributes of doner kebab with the addition of different types, levels and forms of inulin. After that, the panel's acceptability towards this product was tested using sensory evaluation. Due to the labelling requirement of adding new ingredients, an enzymatic method has been used to detect inulin for labelling purposes.

The third and fourth experiments (Chapter 4 and 5) were conducted with fish samples bought from markets in Erbil/KRI aimed at examining the fish authentication in the region. First a molecular DNA barcoding was applied to identify fish identity and detect potential mislabelling issues. The fourth experiment was designed to assess the possibility of fingerprinting compositional profiles and near-infrared spectroscopy for the authentication of origin of wild and farm common carp (*Cyprinus carpio)* based on chemical and mineral compositions. Finally, in chapter six a recap of the general discussion

and conclusions is given with further lines of studies for future works. Figure 1.6 show a schematic outline of the main identified issues and the applied analytical tools.

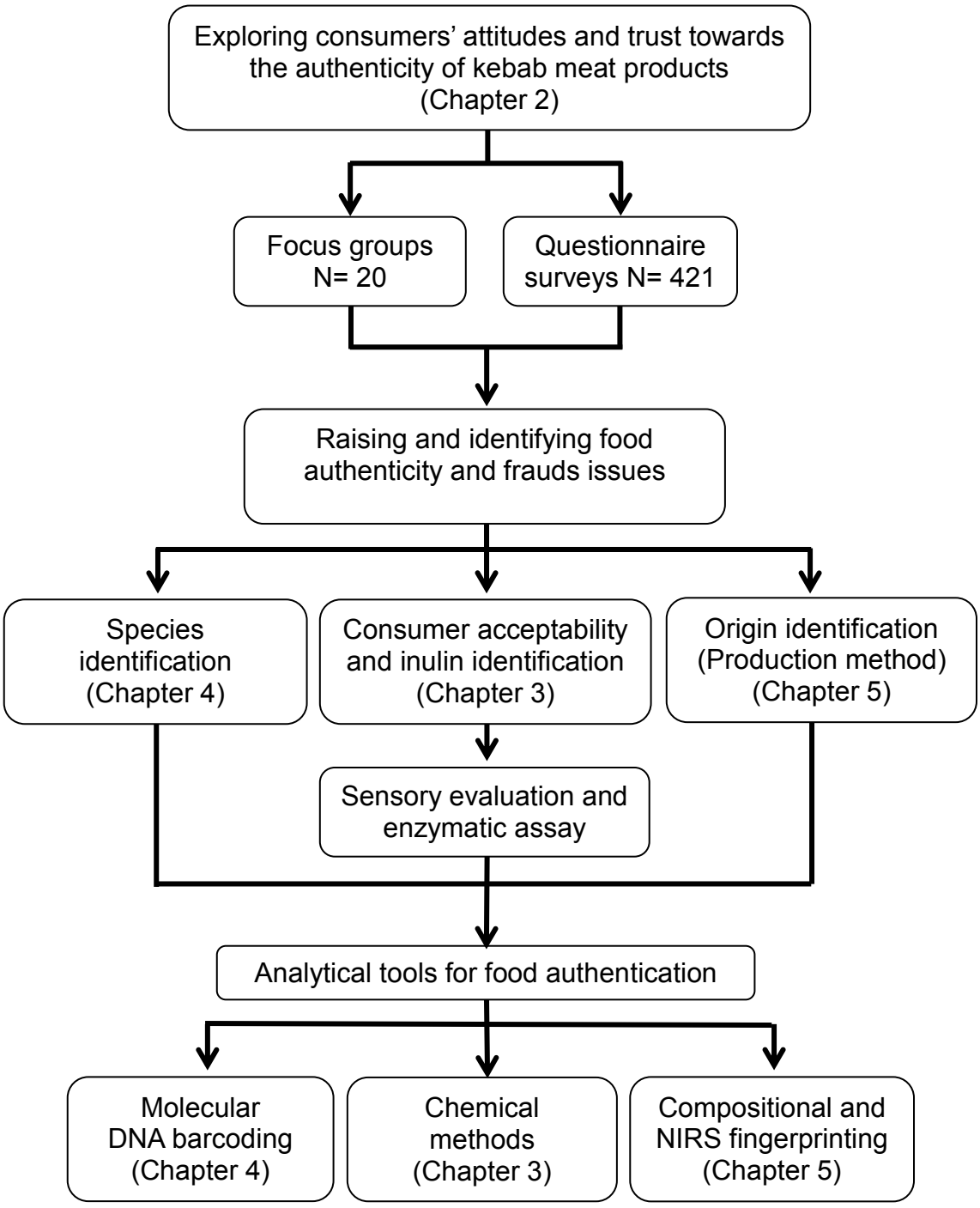


Figure 1.6: A Schematic outline of the main identified issues and the applied analytical tools

1.2.12 The aim and objectives of the study

The overall aim of this study was to explore the consumers' perceptions of food authentication issues in animal products. It is also aimed to develop and apply some analytical tools to identify potential authentication problems of foods from animal products.

The specific objectives of this study were as follows:

1- To investigate the consumer attitudes towards the authenticity of KMP.

2- To identify consumers' main concerns and ways to increase consumer confidence in the meat supply chain.

3- To identify the demographic factors that influences the consumer attitude and trust of KMP.

4- To analyse, determine and compare the cultural orientation (cultural dimensions) of participants in the UK and in KRI and their impact on the dependent variables.

5- To investigate the consumer acceptability of the addition of JA flour and CI as new ingredients for replacing fat in prepared doner kebab.

6- To assess the effects of replacing of doner kebab fat with JA flour and CI, and the effects of the degree of chopping on the cooking characteristics, quality attributes, physicochemical properties and microstructure.

7- To identify and quantify the inulin inclusion on the JA flour, CI, and meat product for labelling purposes.

8- To use DNA barcoding to identify fish bought in Kurdistan markets in order to detect potential mislabelling issues.

9- To determine whether DNA barcoding can be used to distinguish between wild and farmed common carp.

10- To quantify the proximate and minerals composition of muscle fish using NIRS calibration models.

11-To develop robust NIRS calibration models based on the proximate and mineral composition of muscle fish and their NIR spectra to investigate the potential origin discrimination of wild and farmed carp.

12-To measure toxic mineral levels in fish muscle to assess their risk of accumulation in the fish and possibly entry into humans.

Chapter 2

Consumer attitudes and trust towards the authenticity of kebab meat products – Use of qualitative and quantitative approaches

2.1 Introduction

Understanding consumer perceptions of meat and meat products is pivotal for the meat industry due to their direct impact on industry profitability (Troy and Kerry, 2010). Purchasing and consumption of meat is a direct result of how meat is perceived by consumers (Richardson, 1994). Several factors may affect consumers' perceptions, and this depends on the complex interrelationships and interactions between cultural beliefs, the society in which they live, personal factors, and situational factors, such as income and employment status (Axelson, 1986).

Others factors which could determine consumers' perceptions towards shopping behaviour and product choice include price, quality and the value of foods (Zeithaml, 1988). Therefore, consumer perception or food related-behaviour is dynamic, variable (Troy and Kerry, 2010), and complex, and a multidisciplinary approach is needed (Axelson, 1986).

Research on food marketing is essential to provide insight into consumer purchasing patterns (Kilic, 2009). Customer satisfaction of goods or services is the degree of satisfaction measured qualitatively or quantitatively (Kenett and Salini, 2011). For decades, researchers have been using FG as a tool for qualitative research (Morgan, 1997). Furthermore, it can be used as an alternative research method or supplementary source of data in studies that rely on some other primary method, such as a survey (Morgan, 1997, Puchta and Potter, 2004).

Surveys remain a popular channel for data collection in the social, behavioural and consumer sciences, especially after the introduction of the internet, using

WBS (Roztocki, 2001, Weber and Bradley, 2006). Researchers agreed that the introduction of WBS was a significant advance in the field of survey methodology (Couper, 2000, Couper *et al.*, 2001, Crawford *et al.*, 2001, Cobanoglu and Cobanoglu, 2003, Porter and Whitcomb, 2003). Surveys are used to measure customer satisfaction based on the gap between customer expectations and marketing (Kenett and Salini, 2011). Many studies used a combination of FG and questionnaire surveys to explore consumer attitudes toward a particular aspect of food from animal products (Verbeke and Brunso, 2005, Lee *et al.*, 2012, Claret *et al.*, 2014).

Kebab is a traditional Middle East meat product, which is consumed widely in many areas of the world (Kilic, 2003, Kilic, 2009). It is a meat product with fat content ranging between 20-40% (Kilic and Richards, 2003, Kilic, 2009). There are a variety of kebab meat products (KMP) such as doner or shawarma, shish, kofte and mixed doners (LACORS, 2009). For example, doner kebab is reportedly a Turkish national dish (Döner kebab, literally “turning roast”) (LACORS, 2009), slowly roasted on a rotating spit made mostly from intact muscle or ground lamb, beef and chicken meat and is seasoned with onion, tomato, and spices (Kayisoglu *et al.*, 2003, Gonulalan *et al.*, 2004, Kilic, 2009). It is also known by other names such as shawarma or chawarma, donna-kebab and gyro (Kayisoglu *et al.*, 2003).

Kebab meat products are popular in restaurants and fast-food outlets in Middle Eastern countries, Turkey, Europe, Canada, and in the USA (Kilic, 2009). The kebab industry has gained popularity since it was introduced to the UK in the 1960’s (LACORS, 2009). According to the 5th British Kebab Awards 2017, there are over 20,000 Kebab outlets in the UK, selling around 2,500 tonnes of lamb

and chicken doner a week, with an estimated of 1.3 million kebabs sold every day. The British kebab industry contributes over £2.8 billion annually to the British economy, providing around 200,000 jobs across restaurants, suppliers and into the wider food industry in the UK (British Kebab, 2017). KMP are widely consumed in the UK and some other parts of Europe, and are considered one of the fastest growing sectors in the fast food market (Meldrum *et al.*, 2009).

These growing patterns are due to lifestyle changes over the last few decades. Moreover, there is an increasing frequency of meals consumed outside of the home, and even meals consumed at home are often from fast food outlets (Jaworowska *et al.*, 2013).

However, some epidemiological studies have recently reported the link between excessive consumption of processed meat with various diseases (McAfee *et al.*, 2010). This link was particularly associated with consumption of takeaway and fast food (Jaworowska *et al.*, 2013), mainly because of fat content and fatty acid composition (McAfee *et al.*, 2010). Consumers often rely on the mass media and its reliability for relevant information that may influence their purchasing decisions to make informed food purchases. Whether consumers alter their consumption behaviours based on media coverage has much to do with the trustworthiness of the news source and consumers' knowledge. Most often the media portray positive effects of certain food products. However, in the event of food scares, the media influences consumers' decisions by notifying them of the risks and hazards associated with certain foods (Yadavalli and Jones, 2014).

For instance, BSE significantly decreased consumer demand for beef and prices dropped by 40% in the UK (Payne *et al.*, 2009). More recently, due to the horsemeat scandal in Europe, the supply of processed meat products has been the focus of social media conversations (O'Mahony, 2013). These were associated with a drop in consumers' confidence when purchasing processed meat products (Walker *et al.*, 2013). KMP were among these meat products negatively affected recently.

Using multiple methods to investigate the same phenomenon could produce more robust findings than a single method outcome (Davis *et al.*, 2011). To date, reports involving qualitative and quantitative research tools to study perceptions on KMP are rarely available. Therefore, the overall aim of this study was to use a combination of qualitative and quantitative methods to better understand and explore consumers' attitudes and their trust towards the authenticity of KMP. These particular products were selected as a case study because it is considered one of the products vulnerable to substitution and authenticity concerns including species identity, addition of non-meat ingredients and meat ingredients of lower value, as well high level of fat, calories and salt. Furthermore, KMP are one of the most popular meat products in KRI and have also gained popularity in the UK.

The objectives of this study were:

- To investigate consumer attitudes towards the authenticity of KMP.
- To identify consumers' main concerns and ways of helping to increase consumer confidence in the meat supply chain.
- To identify the demographic factors that influences the consumer attitudes and trust of KMP.
- To analyse, determine and compare the cultural orientation (cultural dimensions) of participants in the UK and in KRI and their impact on the dependent variables.

2.2 Materials and Methods

The Human Ethics Committee, Faculty of Science and Environment, Plymouth University, granted ethical forms for this study as a requirement in order to conduct the FG sessions and the WBS.

In this study, two methodologies were used including the FG (qualitative research) and the WBS (quantitative research). Due to the differences between the two tools, the methodologies of both methods have been described separately, while the results have been explained and discussed together in order to find out the effects of both approaches on overall consumer attitudes towards KMP.

Often FG discussions are used as the first stage of investigation followed by quantitative research, such as surveys (Creswell, 2007). The qualitative study was designed to develop of the subsequent quantitative survey. Therefore, in

the present study, FGs were first conducted to formulate the key issues, concepts and questions that would be developed for the WBS, and to provide a useful opportunity to revise and scrutinize the WBS content and structure. A quantitative study is therefore required to confirm the findings from the qualitative approach.

The prepared questions in this study for both tools were derived from past literature reviews, published papers and press in food safety, quality and authenticity where consumers raised their concerns on several issues surrounding the authenticity of KMP from fast food takeaways. These issues mostly concerned species identity, meat content, origin authentication, additional ingredients, high calories, fat and salt content (FSIS, 2007, Askin and Kilic, 2009, LACORS, 2009, Gök *et al.*, 2014, FSA, 2015). Other questions were also included as new ideas linked to consumer perception, authenticity, and how to build and restore consumer confidence in the meat supply chain. All the questions and the options of the questions were directly or indirectly relevant to the aims of this study.

2.2.1 Focus groups (FG)

2.2.1.1 Recruitment procedures and questions content

FG recruitment from within students and staff of Plymouth University was conducted in November 2013 by a direct invitation by e-mail (Appendix 2.1). It included a reference to the topics to be reviewed around one week before each group's meeting. A copy of the briefing information sheet (Appendix 2.2) was attached to the invitation e-mail that clearly explained the participant's right to

withdraw during the FG and at any time after the FG had finished in accordance with the Ethical Form Protocol.

A total of 20 individual participants who expressed their interest in participating in the FG (all meat eaters) were sent a confirmation email with more information about the time and place of the session to be taken (Appendix 2.3). Participants were divided into four groups corresponding to the sessions held within a period of three days. All participants were given the consent information and consent form to read and sign (Appendix 2.4).

In addition to the demographic characteristics of participants, ten questions were prepared for the FG discussions in a form of open-ended questions (Appendix 2.5). Key questions covered the issues of quality attributes and the differences and preferences for doner and shish kebabs. Several questions aimed to discuss the preferred meat species, meat content and ingredients used in KMP. Consumers' trust and their concerns about safety and labelling information on KMP and as well as their willingness to pay for proper labelling were also included.

2.2.1.2 Guide to conducting the focus groups

A guideline was prepared and used to run the sessions (Appendix 2.6). Participants were seated around a conference table in a comfortable room and each session was scheduled to last for approximately one hour. During the introduction, the moderator, assistant moderator and participants began at each session by introducing themselves to other members of the FG. The moderator explained the general nature and purpose of the FG, the ground rules and the

general objective. The discussions were then guided through by a moderator based on the developed set of questions and the guidelines, and all groups covered the same questions. Following the discussion, each participant completed a background questionnaire (Table 2.1), and received a small present for their participation.

2.2.1.3 Data collection and analysis

Qualitative data were generated through group discussions, tasting sessions, and interaction within group activities. Statements made by each participant during the session were manually transcribed verbatim and the conversations were digitally voice recorded. To ensure reliability and validity, data were collected from multiple sources including audiotapes, notes taken by assistant moderator and items recalled by the moderator and assistant moderator (Onwuegbuzie *et al.*, 2009). The qualitative data were started by collecting, preparing and organising the data, and then reducing the data into themes through a process of coding and finally representing the data in figures, tables, or a discussion (Creswell, 2007).

All four sessions were audio taped and then transcribed for further analysis. The collected data were analysed based on individual data, group data, and/ or group interaction data as recommended by Onwuegbuzie *et al.* (2009). Large amounts of data were generated in all sessions; therefore, getting rid of irrelevant information was necessary (Rabiee, 2004). Moreover, the most regular terms used in the results of qualitative data were ‘many’, ‘most’, ‘frequently’, ‘several’, ‘never’, and so on (Onwuegbuzie *et al.*, 2009).

2.2.2 Web-based survey (WBS)

2.2.2.1 Recruitment procedure

Recruitment for the two WBS was conducted through the use of two sources of web invitations. To recruit participants for the WBS in the UK, some students and staff of Plymouth University were asked via an invitation email to participate in the WBS with information sheet (Appendix 2.7), while the Kurdish WBS was posted on Facebook with the web server that contains the consent form and information sheet, targeted at people in KRI, and shared on different social and food related pages (Appendix 2.8).

Both sources of invitations contained the briefing information sheet with a brief explanation of the main topic and clearly explained the participants' right of confidentiality and to withdraw, in accordance with the Ethical Form Protocol. To ensure the anonymity of the respondents, no personal information (e.g. names, addresses, and phone numbers) were collected. The invitations also contained the website address (URL) that automatically logged them into the survey.

2.2.2.2 Web-based surveys design and questionnaire structure

The two WBSs were designed using online survey software (SurveyMonkey, https://www.surveymonkey.co.uk/. The core WBS was written in the English language for the participants in the UK (Appendix 2.9), while the same WBS version was translated into the Kurdish language (excluding first two questions) for the participants in KRI (Appendix 2.10).

From the twenty questions developed, most of them were ranked questions based on specific rating scales, while a few questions were multiple choice

questions, and there was one close-ended question. The number and type of questions selected in the questionnaire were chosen to enable it to be answered within 10-12 minutes (Appendix 2.9). Generally thirteen minutes or less is considered as an ideal length to obtain a good response rate (Fan and Yan, 2010). The questions for WBS were designed to cover topics in Box 2.1

Box 2.1: Topics or themes used for designing WBS

- Demographic characteristics of the respondents including age, gender, cultural groups, education levels and employee status.
- Frequency of eating and spending money on KMP
- Consumer trust in certain fast food products
- Quality attributes and labelling information on KMP
- Consumer knowledge on meat species, content, and other ingredients in KMP
- Consumer reactions towards additional ingredients, including fibres (inulin)
- Consumer feeling on undeclared species and other ingredients
- Impact of the horsemeat scandal on purchasing KMP

2.2.2.3 Sample description

Both WBS were available online for response collection for about six weeks from the end of November 2013 to the early January 2014. A total of 421 participants responded to the WBS in the UK and in KRI, of which 70% were male and 30% were female (Table 2.2). English WBS targeted participants in the UK (n= 241) who were asked to identify themselves into one of four cultural groups; British/European (B-EU), Middle Eastern (ME), Kurdish (Krd) and other cultural background (OCB). The Kurdish WBS targeted residents of KRI (n= 180). The demographic characteristics of the respondents in both WBS are summarized Table 2.2 in results and discussion section.

2.2.2.4 Reliability and validity of web-based survey

Reliability and validity are important tools to support and strengthen research as it is aimed to eliminate the bias and increase the research's truthfulness (Golafshani, 2003). Therefore, as a step towards validation, both questionnaires were first reviewed by an experienced advisor, and comments acted upon to improve the questions and eliminate any ambiguous items. After that, the preliminary versions of both WBS were pilot-tested with 5 respondents, with different demographic profiles, to test the wording adequacy and understanding and the suitability and appropriateness of the formulated questions in relation to the objectives of study.

Observations which could be used to eliminate any potential problem were recorded. The pilot test uncovered a few issues regarding question length and wording, and minor adjustments were made before conducting the actual surveys. For example, some questions were omitted from the questionnaire,

and the options of some multiple questions were kept shorter in order to keep the time for responding to the questionnaire shorter (no more than 12 minutes). Some ambiguous questions and words were also eliminated.

2.2.3 Statistical analysis

Statistical Package for the Social Science (IBM SPSS Statistics for Windows, Version 22, Portsmouth, UK) was used to convert text data from questionnaire answers to numeric data. A number of descriptive procedures were used, including frequencies and cross-tabulations. Chi-square test was used to examine the relationship between some relevant variables. The Friedman test as a non-parametric test was also used to compare the ranking of each variable and to find out whether if there are an overall differences between variables.

2.3. Results and Discussion

2.3.1 Demographic characteristics

In this study a combination of two approaches was used and the data were collected using both FG and WBS. With respect to the demographic characteristics of the respondents, data on gender, age, cultural groups, education level, employee status and period living in the UK were collected, as summarized in Table 2.1 and Table 2.2 for FG and WBS respectively. The impact of these independent variables on the dependant variables (frequency of eating and spending money on KMP, consumer trust on certain food products and the addition of ingredients list etc.) were evaluated.

Table 2.1: Demographic profile of FG participants (n= 20)

Group No.	Respondents code	Gender	Cultural background	Period living in the UK	Age
Group A	A1	F	Other	6-11 months	42
	A2	F	Kurdish	1-3 years	33
	A3	M	Other	3-5 years	32
	A4	M	Kurdish	3-5years	29
	A5	M	Other	6-11 months	36
Group B	B1	F	British/ European	> 5 years	23
	B2	M	Middle Eastern	3-5years	29
	B3	M	Middle Eastern	1-3 years	27
	B4	M	Kurdish	1-3 years	29
	B5	M	Kurdish	1-3 years	38
	B6	F	British/ European	> 5 years	27
Group C	C1	M	Middle Eastern	3-5years	46
	C2	M	Middle Eastern	3-5years	39
	C3	F	British/ European	3-5years	22
	C4	M	Kurdish	3-5years	37
Group D	D1	M	Middle Eastern	> 5 years	31
	D2	M	Kurdish	1-3 years	33
	D3	M	Middle Eastern	> 5 years	70
	D4	M	Kurdish	3-5years	48
	D5	M	Middle Eastern	3-5years	51
Overall (%)		F= 25 M = 75	British/ European =15 Middle Eastern = 35 Kurdish = 35 Others = 15	6-11 Month=10 1-3 Years= 25 3-5 Years= 45 > 5 Years= 20	Mean=36 SD=11.25

F= female, M= Male

Table 2.2: Distribution of characteristics for English and Kurdish respondents of the WBS (N= 421)

Characteristics	English WBS (n= 241) (%)	Kurdish WBS (n= 180) (%)
Gender		
Male	69.3	70.5
Female	30.7	29.5
Age groups		
19-25	15.7	35
26-30	34.8	36
31-35	31.5	15
36-40	9	6
Over 41	9	8
Cultural groups		
British/ European	19	All from KRI
Middle Eastern	37	
Kurdish	31	
Others	13	
Period being in the UK		
< 6months	10.3	N/A
6 Months to 11 Months	11.2	
1-3 Years	37.8	
3-5 Years	15.8	
> 5 Years	24.9	
Employee status		
Student	75	22.2
Employed	21.5	67.3
Unemployed	3	8.3
Retired	0.5	2.2
Education level		
High school or below	0.8%	7.2
College/ University	29.5%	54.4
Graduated	69.7%	38.4

2.3.2 Consumer preferences and trust of kebab meat products

Often FG is a good tool to begin an investigation as it reflects points of view, and is useful for exploring people's knowledge and experiences. It can be used to examine not only what people think, but how they think, and why they think that way (Kitzinger, 1994). Therefore, the present consumer research study began with the FG sessions and with general questions about familiarity, trust and preference among KMP.

The aim of these basic questions was to aid group discussion and to gain a better understanding of consumers' attitudes towards KMP. Consumer trust of KMP meant the overall trust of the products such as the composition (meat content, species, and any other ingredients), hygienic conditions of the products, kebab shops and personal hygiene.

Most participants in the FG showed their familiarity and great interest for the topic by their effective involvement during the discussions. Their interest in the topic could be due to the popularity of the KMP in restaurants and fast-food outlets in many countries and within cultural backgrounds (Kilic, 2009). The majority of participants agreed that they preferred, trusted, and were more satisfied with shish kebab than doner kebab (Question1, Appendix 2.5), and the reasons given by participants are listed in the box 2.2. Respondents in both WBS were also asked about their trust of certain types of fast food products, including KMP (Question 10, Appendix 2.9).

Box 2.2: Typical comments by participants about their preferences towards KMP in the FG with their respondent's code*

- "Prefers shish kebab to other fast foods, wants to be able to trace kebab meats" [A1]
- "Both are unhealthy if you eat frequently, loves doner more than shish kebab" [A2]
- "Prefers and trusts shish kebab more than doner, feels that doner kebabs are intimidating" [B1]
- "Prefers and trusts shish kebab as they are tastier and healthier more than doner" [B3]
- "Doesn't know the differences between shish and doner kebabs" [C3]
- "Prefers and trusts shish kebab more than doner because it is cooked over charcoal while the doner has too much fat" [C4]
- "Very familiar with both products, has a couple of times a month but doesn't trust either" [D1]
- "Big fan of shish kebab and eat 1-2 a week, doesn't like doner kebabs as I don't know how they prepared" [D4]

*These codes can be found in Table 2.1 for further demographic characteristics for each participant

Participants show their degree of trust from more trust to distrust in both countries (Table 2.3 and 2.4). Pizza, fish and chips, and vegetable burgers were the top three most trusted products, while beef burgers and sausages were the least trusted products in both countries. One reason for the lower trust among respondents for fast food with meat products (sausages, beef burgers and

kebab products) compared to pizza, vegetable burgers and fish chips (Table 2.3 and 2.4) could be due to the food scandals and scares related to meat products. Often consumer concerns increase with every food scandal and scare, which is where trust and distrust have been seen to arise. Indeed, different food scandals highlight different dimensions of trust (Kjaernes *et al.*, 2007). Furthermore, the respondents' degree of trust in sausages in the UK, and in sausages and beef burgers in KRI was low. This has also been explained in the waterfall charts (Appendix 2.10) based on the obtained mean of rating scales (Table 2.3 and 2.4). Regarding the differences between shish and doner kebabs, respondents in both countries had more trust in shish kebab than in doner kebab, as observed in FG discussions.

Table 2.3: Ranking the degree of trust in fast food products in the UK (n=241)

Fast Foods	**Degree of trust (Mean± SD)***	**Mean rank[×]**
Pizza	3.68±1.00	5.59
Fish and chips	3.49±1.19	5.23
Vegetable burgers	3.49±1.13	5.18
Shish kebab	3.47±1.00	5.15
Pasty	3.09±1.10	4.36
Doner kebab	2.95±1.11	4.15
Beef burgers	2.86±1.15	3.78
Sausages	2.16±1.18	2.56

*Rating scales were 1=no trust at all, 2= some distrust, 3= neutral, 4= some trust, and 5= full trust. [×]Mean rank was calculated based on the respondent rating scales given to each variable (1-5), and then these rating scales were ranked for each variable within one respondent. The mean rank was obtained from the sum of rank of each variable of all respondents then divided by the number of all variables.

Table 2.4: Ranking the degree of trust in fast food products in KRI (n=180)

Fast foods	Degree of trust (Mean± SD)*	Mean rank
Fish and chips	3.74±1.23	5.53
Vegetable burgers	3.26±1.25	4.74
Pizza	3.16±1.18	4.57
Shish kebab	2.83±1.24	4.02
Doner kebab	2.57±1.22	3.61
Beef burgers	2.20±1.03	3.01
Sausages	1.90±1.01	2.51

To test the significance of the impact of cultural groups on respondents' trust, a cross-tabulation table was conducted. Cultural groups in the UK are significantly linked ($p< 0.05$) to respondent trust for all products (except for vegetable burger and shish kebab) and highly significantly linked ($p< 0.001$) to trust in sausages (Appendix 2.11). For example, British/ European participants tend to place more trust in pizza, fish and chips, beef burgers, sausages and pasties compared to the other three groups in the UK. This could be due to their familiarity with these products as traditional fast foods. Similar trends were also observed with other cultural groups compared to Kurdish and Middle Eastern groups, whereas the distrust in sausages and beef burgers was more noticeable in Kurdish and Middle Eastern respondents (Figure 2.1). Although sausages are popular meat products with millions of consumers around the world, this distrust could be due to the increasing concerns about the potential health risk associated with the consumption of high-fat foods (Mendoza *et al.*, 2001).

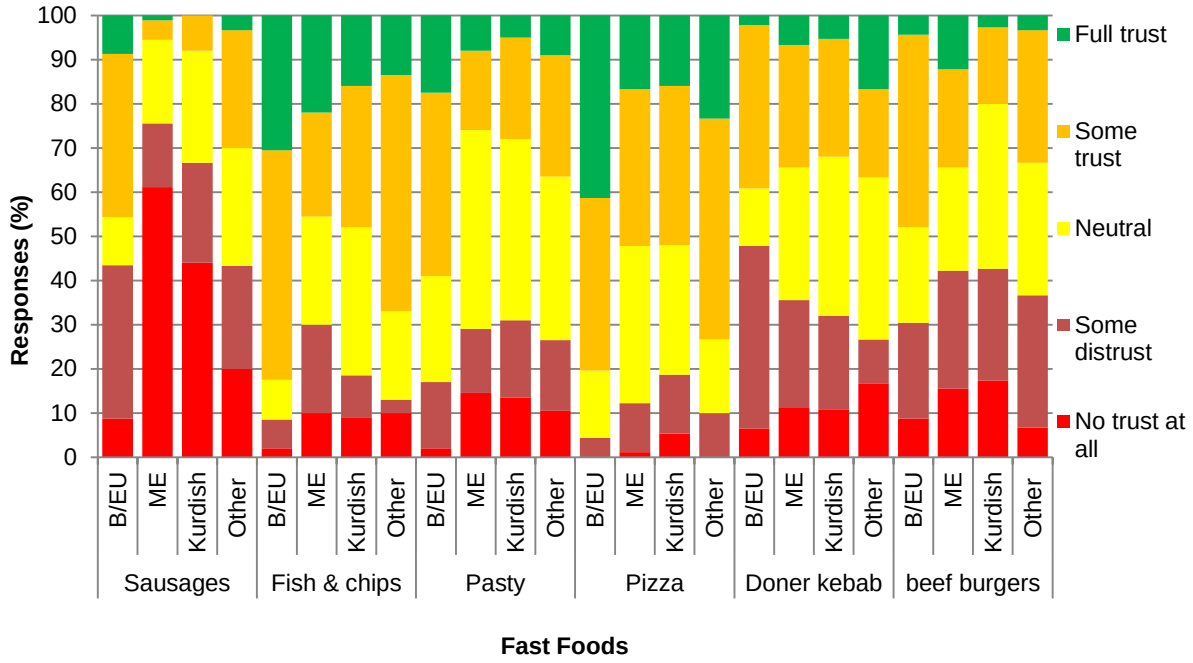


Figure 2.1: Respondent trust among cultural groups in the UK for certain products. B/EU= British/ European and ME= Middle Eastern

Gender only had a significant ($p < 0.05$) impact on respondent trust in shish kebab in the UK. For instance, males tend to trust shish kebab more than females, while the significant effects ($p < 0.05$) of gender on respondents in KRI was only on pizza where females had more trust (Appendix 2.11).

Age has been shown to be a significant variable in participants' trust. Different age groups in the UK seemed to have significant ($p < 0.05$) differences in trust for sausages, vegetable burgers, and shish kebab and pizza. For instance, respondents over 40 years old are more trusting, and respondents who lived in the UK for more than 5 years tended to be significantly ($p < 0.05$) less distrusting in sausages (Appendix 2.11).

Sausages were more trusted by British/European respondents, especially by those over 41 years old, while the same products were significantly less trusted

by respondents in KRI within the same age group (over 41). Actually, 40 % of the Kurdish participants in the UK distrust sausages. Education level and employment status in both countries had no significant ($p > 0.05$) effect on respondent trust.

Regarding the second question in the FG, “Why do you eat KMP in comparison to other fast foods?” (Question 2, Appendix 2.5), most participants (90%) preferred KMP over other fast foods for different reasons which ordered from more important to less important reasons as follows; taste, open late availability, easy to find halal KMP, reminder of home food and costing less. In addition to the taste as the most important factor to prefer KMP, open late availability was the second most driving factors that drew participants to prefer KMP compared to other fast foods. A survey conducted by Chalak and Abiad (2012) also found that location parameters had significantly ($p < 0.05$) affected the average households preference to buy shawarma sandwiches (doner kebab) from local shops (around the corner). Furthermore, doner kebab gradually became a popular fast food in Britain, particularly when it comes to what to eat after a night out (Sirkeci, 2016).

Similar results were observed in the WBS that the consumption of KMP was more frequent than beef burgers. For example, respondents in both WBS were asked whether they ever ate certain types of meat products, including shish kebab, doner kebab and beef burgers (Question 7, Appendix 2.9). Results show that around 90% of participants in both countries ate KMP (doner and shish), while only about 70-80% have tried beef burgers (Table 2.5).

Table 2.5: Consumption patterns of three types of meat products in the UK and in KRI

Meat products	UK (n=241)		KRI (n= 180)	
	Eaten	Never eaten	Eaten	Never eaten
Doner kebab	91.29%	8.71%	89.44%	10.66%
Shish kebab	92.53%	7.47%	94.4%	5.6%
Beef burgers	80.91%	19.09%	70%	30%

There was a significant difference ($p < 0.05$) between cultural groups and eating of shish kebab, and beef burgers. For example, when the cultural groups in the UK were compared (Figure 2.2), more than 95% of the participants in British/European group were found to eat beef burgers. In contrast, the other three groups, especially the Middle Eastern group, were found to eat more KMP than beef burgers.

This consumption pattern can be linked to the cultural impact on decision making. For example, according to Chambers *et al.* (2007), consumer choice can vary depending on demographic characteristics. Ethnicities play an important role in consumer demand for goods. Usually, areas with more diverse populations are associated with a variety of food products (Resurreccion, 2004), and have brought with them their own food cultures (Khokhar *et al.*, 2013). KMP as a traditional Middle Eastern product (Kayisoglu *et al.*, 2003), was eaten more often by Middle Eastern, Kurdish and other cultural groups compared to British/European participants.

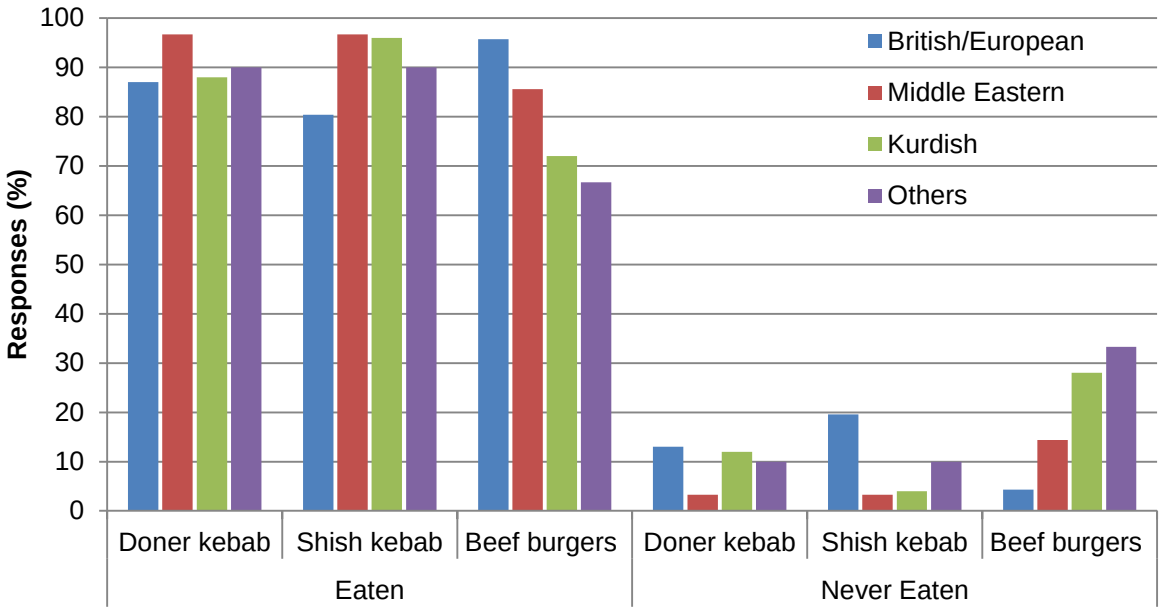


Figure 2.2: Consumption patterns of three types of meat products among different groups in the UK (n=241).

Furthermore, there was only a significant difference ($p < 0.05$) (Appendix 2.11) in the UK between gender and the eating of beef burgers, and interestingly about 92% of females had eaten beef burgers compared to only 76% of males. Moreover, male respondents in KRI tended to eat shish kebab more often ($p < 0.05$) than females. Age groups for respondents in KRI had statistically significant differences ($p < 0.05$) for consumption of doner kebab, where younger groups tended to eat doner kebab more than participants aged over 36 years old, while age groups in the UK had no significant difference (Appendix 2.11).

In addition, respondents were questioned in the WBS (Question 8 and 9, Appendix 2.9) about their frequency of eating and spending money on KMP in both countries. In general, results showed that participants in KRI tended to eat

more frequently than respondents in the UK (Figure 2.3). Similar results were also obtained in terms of spending money on eating KMP in both countries (Figure 2.4).

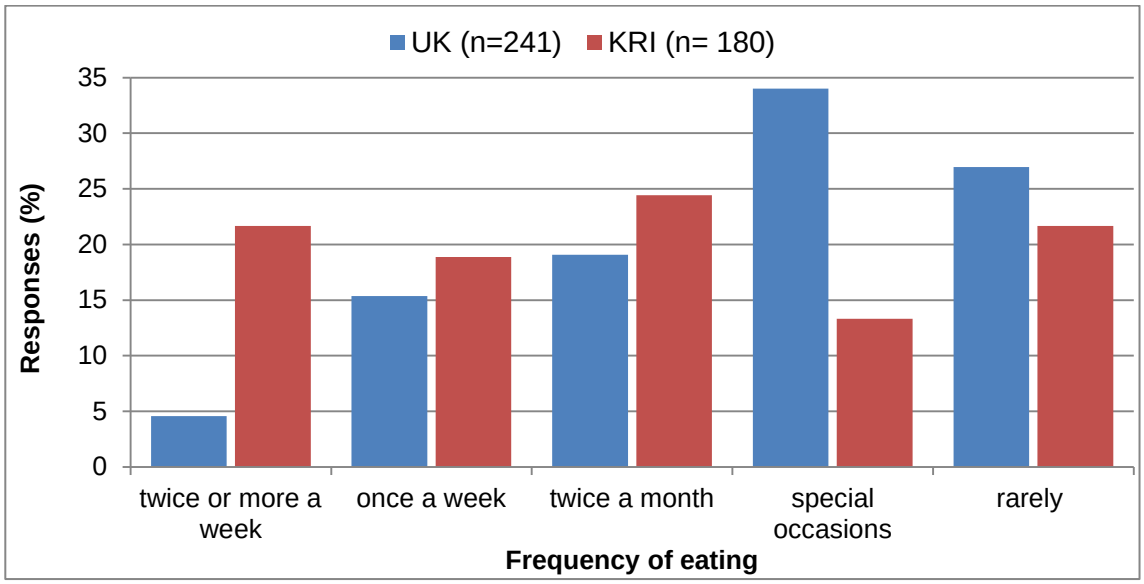


Figure 2.3: Frequency of eating KMP in the UK and in KRI

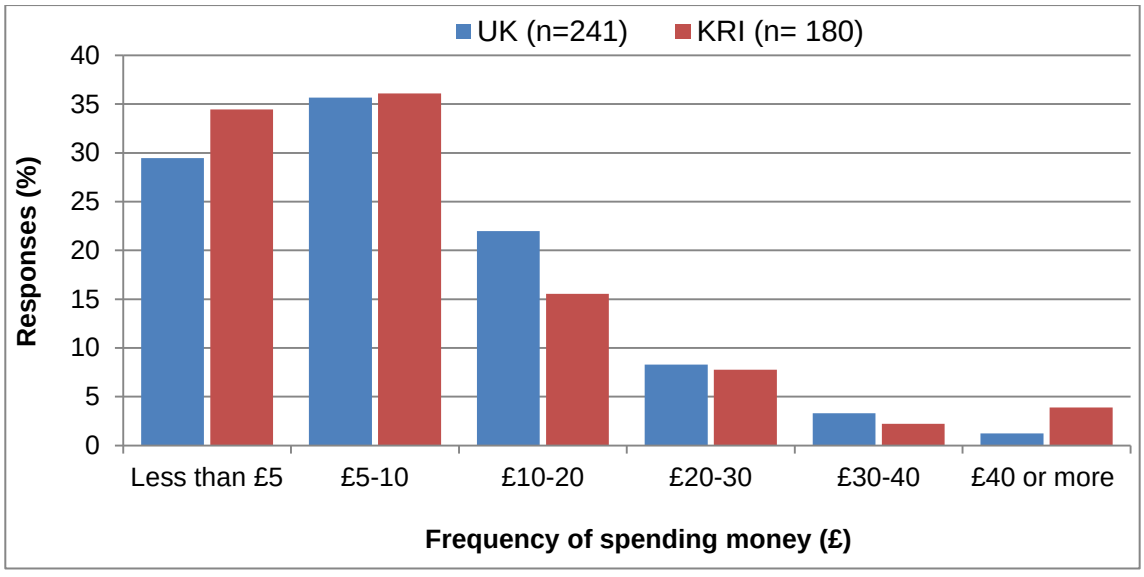


Figure 2.4: Frequency of spending money on KMP in the UK and in KRI

For example, 65% of participants in KRI tended to eat KMP at least twice a month compared to only 39% for the UK respondents. Approximately 61% of participants in the UK eat KMP either on special occasions or rarely (Figure 2.3). According to the results of cross tabulation (Appendix 2.11), there was a highly significant ($p < 0.001$) difference between cultural groups in the UK and the frequency of eating KMP. The frequency of eating KMP among investigated groups in the UK from more to less frequently were found as follows; Kurdish, Middle Eastern, other culture and British/ European respectively (Figure 2.5).

This trend can reflect respondents' perception of their traditional meals of their original destinations as explained by Sirkeci (2016), who confirmed that when consumers change their place of residence or are involved in commuting, they often demand the same products and services at their destinations or new homes. Over time these types of food products such as döner Kebab may gain substantial market share and become recognised as part of the national food culture.

However, when the Kurdish participants compared in both countries, Kurdish participants in the UK tended to eat KMP less frequently than Kurdish participants in KRI (Figure 2.6). This is an indication that consumer perceptions of the Kurdish participants have changed since their coming to the UK.

According to a UK survey of adults conducted in 2002, there was a 10% increase in the buying of take-away food from doner kebab outlets between 1999 and 2002 (Mintel Market Intelligence, 2002). This increase was also demonstrated in this study, especially with Middle Eastern and Kurdish respondents in the UK.

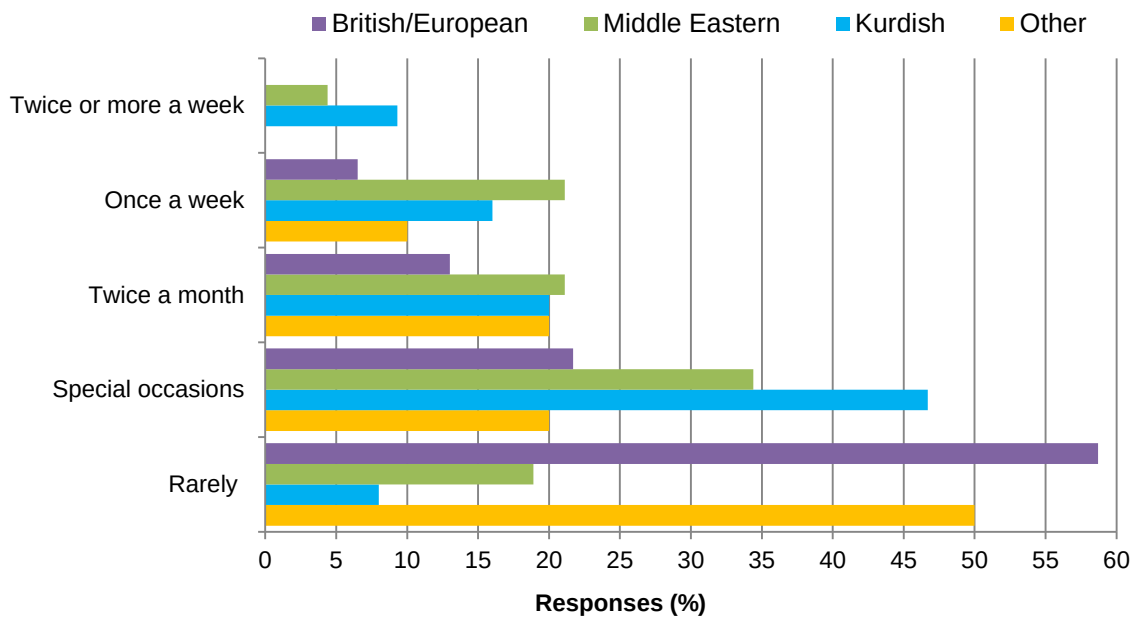


Figure 2.5: Frequency of eating KMP among cultural groups in the UK (n=241)

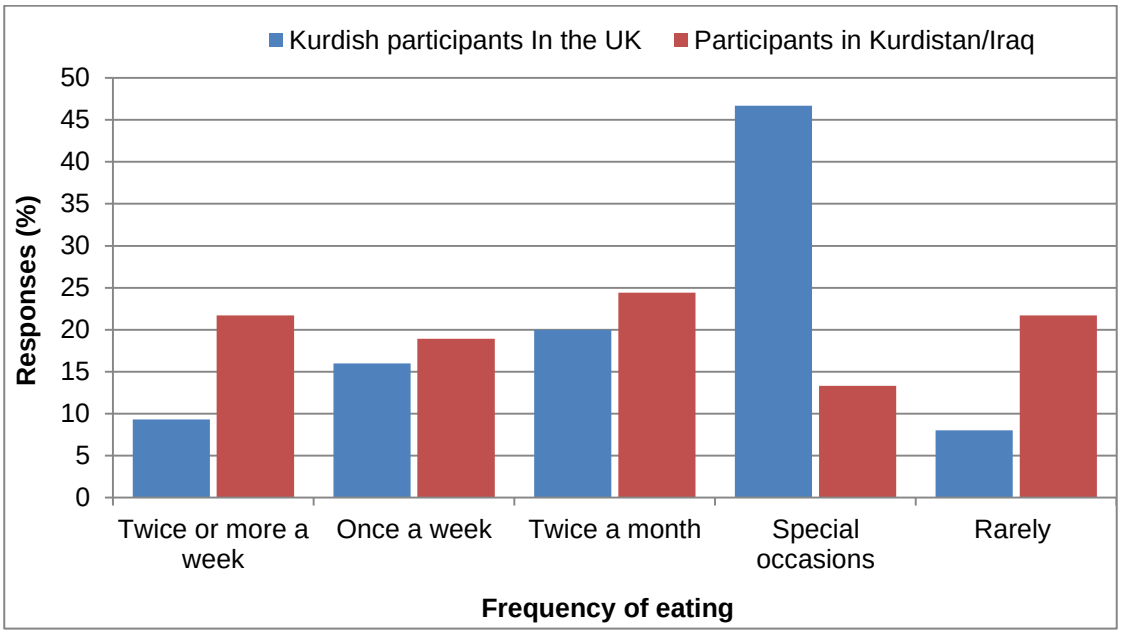


Figure 2.6: Frequency of eating KMP of Kurdish respondents by country of residence (UK and KRI)

The statistical cross tabulation found significant ($p < 0.05$) differences between period resident in the UK and the frequency of eating KMP. In this case, participants who lived less than 6 months in the UK (mostly Middle Eastern, including Kurdish participants) had more frequently eaten KMP compared to other participants who lived in the UK for longer. It may be that consumer perceptions may take longer than a year to adapt to new environments and cultural orientation.

Although food choices can vary, existing research suggests that demographic characteristics act as a function for decision making. For instance, women tend to have different diets from men (Bates *et al.*, 1999, Fagerli and Wandel, 1999). In this regard, gender has been shown to be a significant variable in the frequency of eating KMP in the present study. Male participants tended to eat KMP significantly ($p < 0.05$) more than females in both countries (Appendix 2.11).

Similar trends were also found by Beardsworth and Bryman (1999) and Beardsworth *et al.* (2002), who found that men eat meat more often than women. There is much evidence world-wide that males and female do not consume equal allocations of foods. This could be due to the average differences in weight between male and female, for which men would require more meat than women, rather than physiological reasons (Gossard and York, 2003). Frequency of eating KMP was significantly affected by age group only in the UK. The first three age groups (19-25, 26-30 and 31-35) ate KMP more frequently than people aged over 36 years (Figure 2.7 and 2.8). Similarly, Beardsworth *et al.* (2002) found that 47.5% of respondents indicated they ate less red meat compared with ten years ago when they were younger, and this

was more significant in women than in men. This could be due to the warnings from published papers who recommended moderate consumption of fresh red meat and the avoidance of processed meat products as desirable for the prevention of some chronic diseases (Verbeke *et al.*, 2010).

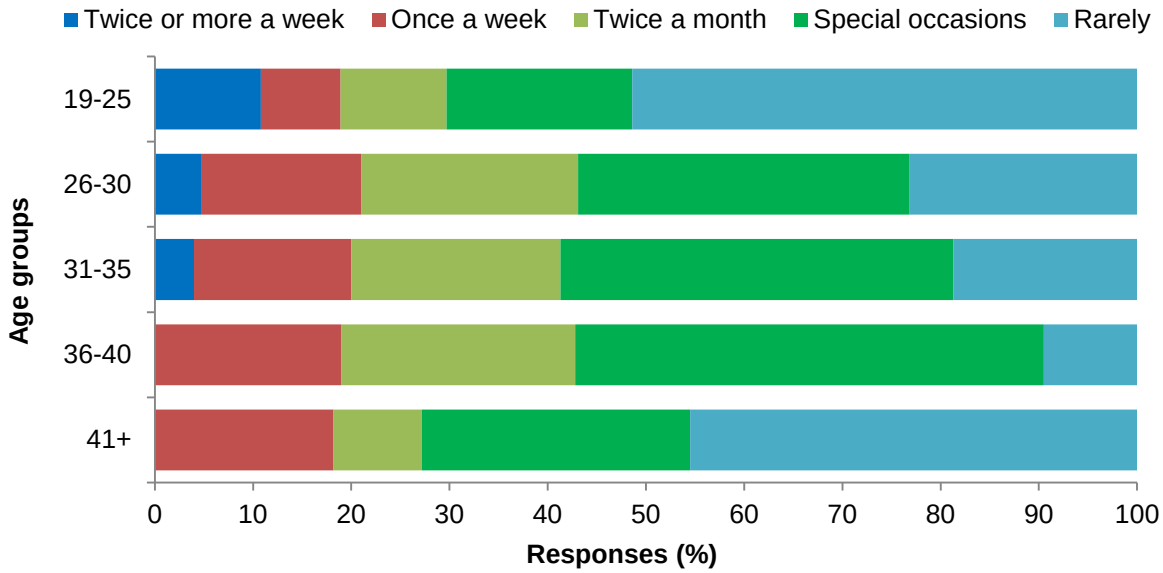


Figure 2.7: Frequency of eating KMP among age groups in the UK (n=241)

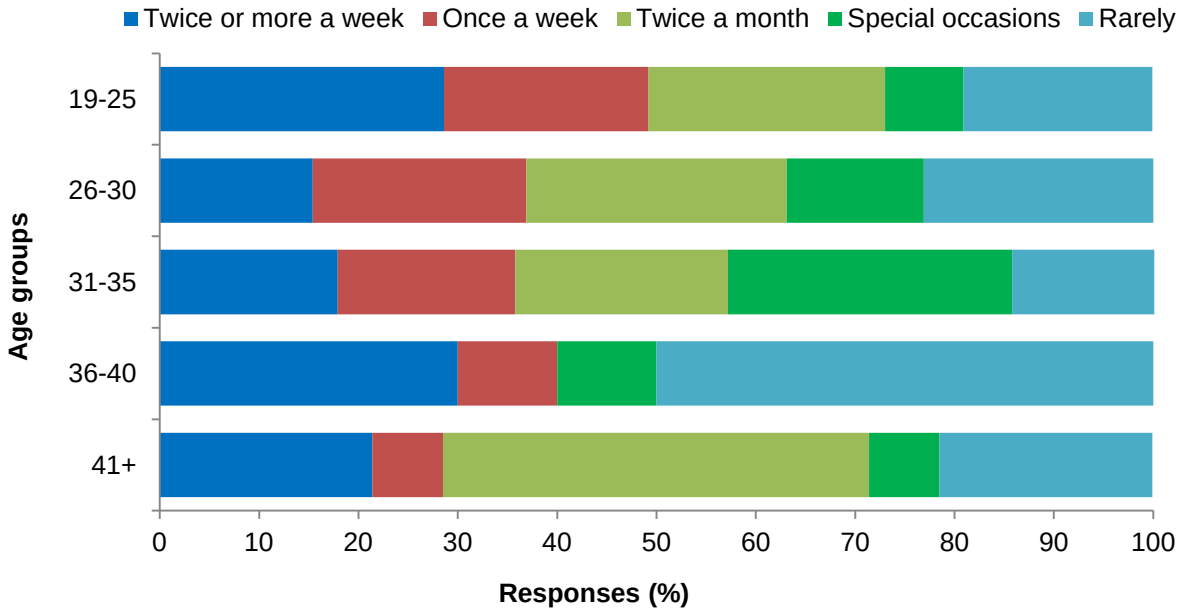


Figure 2.8: Frequency of eating KMP among age groups in KRI (n=180)

The factors that most influenced participants' decisions to purchase KMP in the FG were taste, open late availability of kebab shops, and affordable price (Question 3, Appendix 2.5). A qualitative study consisting of a series of FG on beef steak conducted in four European countries, including the UK, also found that good flavour was among the top quality attributes (Grunert, 1997).

Regarding the quality attributes of KMP, nineteen attributes of KMP in both WBS (Question 11, Appendix 2.9) were summarised and ranked for their degree of importance when purchasing KMP, from most to least important (Table 2.6 and 2.7). Although there were some differences between the degrees of importance of the quality attributes in both countries, the results indicated that the top five quality attributes for both WBS were taste, safety of meal, halal/kosher, being freshly made, and flavour.

The most obvious difference between the respondents in both countries was the halal/ kosher attribute, which was the fifth most important attribute to purchase KMP in the UK, while halal attributes was the most important attribute for respondents in KRI. This can be explained by kebab outlets mostly using halal supplies (Sirkeci, 2016) It is also linked to the cultural belief orientation. For example, Richardson (1994) pointed out that the perceptions of foods are also based on personal experience, the experience of friends, family and media channels. Furthermore, religion as culture can also play a role in food preferences (Tiu Wright *et al.*, 2001).

For instance, Muslim populations choose to avoid pork and pork products in their foods (Premanandh, 2013). Chalak and Abiad (2012) reported that consumer decision making and choice behaviour are often influneced by

different attributes including intrinsic (e.g., taste, texture, colour), or extrinsic(e.g. label or brand and packaging).

Respondents were also asked to rate some statements asked in the WBS (Question 20, Appendix 2.9) to what extent they agreed or disagreed. “I never worry about the quality of kebab meal”, obtained results showing that UK respondents were more worried about the quality of KMP. For instance, about 40% of UK respondents (Table 2.14) were strongly worried about the quality compared to only 29.5% in KRI (Table 2.15). This could be due to the fact that respondents in the UK were more conscious about the quality of meat products compared to those in KRI.

Table 2.6: Ranking the degree of importance of the quality attributes of KMP in the UK (n=241)

Attributes	**Degree of importance (Mean± SD)***	**Mean rank**
Taste	3.66±0.59	14.13
Safety of meal	3.56±0.72	13.66
Freshly made/ freshness	3.49±0.71	13.16
Flavour	3.47±0.64	12.83
Halal/ kosher	3.14±1.22	11.52
Past expeience	3.17±0.81	10.96
Overall apprearance	3.11±0.76	10.71
Authentic/ origin	3.10±0.82	10.57
Convenience	3.06±0.78	10.27
Composition of meal	3.00±0.84	10.01
Price	2.87±0.78	9.19
Tenderness	2.84±0.83	8.89
Aroma	2.83±0.83	8.82
Nutritional value	2.80±0.92	8.72
Wholesomeness	2.68±0.89	8.07
Following friends and family	2.60±0.91	7.80
Trying something different	2.62±0.85	7.77
Special occasion	2.56±0.93	7.60
Fashion	2.14±0.91	5.29

*Rating scales were 1=not important, 2= little important, 3= somehow important, and 4= most important.

Table 2.7: Ranking the degree of importance of the quality attributes of KMP in KRI (n=180)

Attributes	Degree of importance (Mean± SD)*	Mean rank
Halal/ kosher	3.87±0.44	14.35
Safety of meal	3.72±0.62	13.44
Taste	3.70±0.55	13.16
Freshly made/ freshness	3.62±0.69	12.73
Flavour	3.60±0.61	12.58
Composition of meal	3.42±0.71	11.32
Overall apprearance	3.27±0.86	10.63
Aroma	3.26±0.79	10.51
Convenience	3.18±0.89	10.03
Wholesomeness	3.09±0.85	9.47
Tenderness	3.08±0.78	9.32
Trying something different	2.97±0.85	9.07
Nutritional value	3.03±0.88	8.99
Special occasion	2.87±0.96	8.45
Price	2.81±0.98	7.90
Fashion	2.77±1.02	7.82
Past expeience	2.70±0.96	7.48
Authentic/ origin	2.65±0.91	6.98
Following friends and family	2.37±1.00	5.77

*Rating scales were 1=not important, 2= little important, 3= somehow important, and 4= most important.

2.3.3 Labelling declaration of ingredients in KMP

The quantity of meat in a meat product by species (e.g. beef %, lamb %) is essential for informing consumers (FSA, 2003, FSIS, 2007). Generally the calculation of meat content by manufacturer can follow one of three separate methods, including the FSA method, the CLITRAVI method (Liaison Centre for the meat processing industry in Europe), and Stubbs and more calculation method (FSIS, 2007).

The QUID is a legal requirement applied to meat products sold pre-packed, loose, or pre-packed for direct sale from a retail outlet (i.e., butchers, bakers, delicatessen counters etc.). However, QUID does not apply to meat products sold loose or pre-packed for direct sale from catering establishments including restaurants, canteens, clubs, public houses, schools, hospitals or similar establishments. Nevertheless, retailers are free to provide QUID declarations for certain exempt meat products or ingredients on a voluntary basis (FSA, 2003).

For example, when catering services are supplied with manufactured KMP, the meat quantity should be provided. However, when the final product is sold to the consumer there is no requirement to provide meat content. Moreover, the FSA have advised that a meat product named simply “Doner kebab” can be considered a customary name for a sheep meat product containing only lamb or mutton unless further qualification is provided (LACORS, 2009). However, it is against the law to mislead consumers and fines up to £5000 can be imposed under EU Regulation 178/2002.

In this context, participants were asked several questions in both FG and WBS to assess their awareness and preference of the types of meat species, the levels of meat content and other ingredients that may be involved in manufacturing KMP. For instance, the observed data in the FG discussions (Question 5, Appendix 2.5) indicated that the majority of participants preferred lamb meat followed by chicken meat. In the FG discussions, chicken meat was preferred by other cultural groups in the UK, while all the British/European participants preferred lamb. Most of the participants preferred KMP with one species rather than mixed species.

Similarly, a question was asked of participants in the WBS (Question 17, Appendix 2.9) to indicate their preferred and unexpected meat species in KMP. Results showed that the preferred meat species were lamb, chicken, beef, mutton, goat, and fish consecutively, while pork and horse were unexpected to be part of KMP in the UK. This was in line with the fact that doner kebabs are generally made mostly from intact muscle or ground muscle of lamb, chicken and beef meats (Gonulalan *et al.*, 2004, Kayaardi *et al.*, 2006, Kilic, 2009). Furthermore, lamb is the most common meat in KMP in the UK, whereas beef is dominant in Germany and continental Europe (Sirkeci, 2016).

For example, about 85% of participants in the UK indicated their preference for lamb and chicken meat compared to only about 15% of participants who did not expect lamb and chicken to be a part of KMP (Figure 2.9). Furthermore, when the cultural groups in the UK were compared, about 90% of participants preferred lamb meat as the first choice. Exceptions were mainly participants of other cultural backgrounds, where they preferred chicken as the first choice, as observed in the FG (Table 2.8). Respondents from KRI had similar preferences,

but more towards lamb, chicken, mutton and then beef. Therefore, participant's responses in both tools had similar preferences for lamb meat followed by chicken.

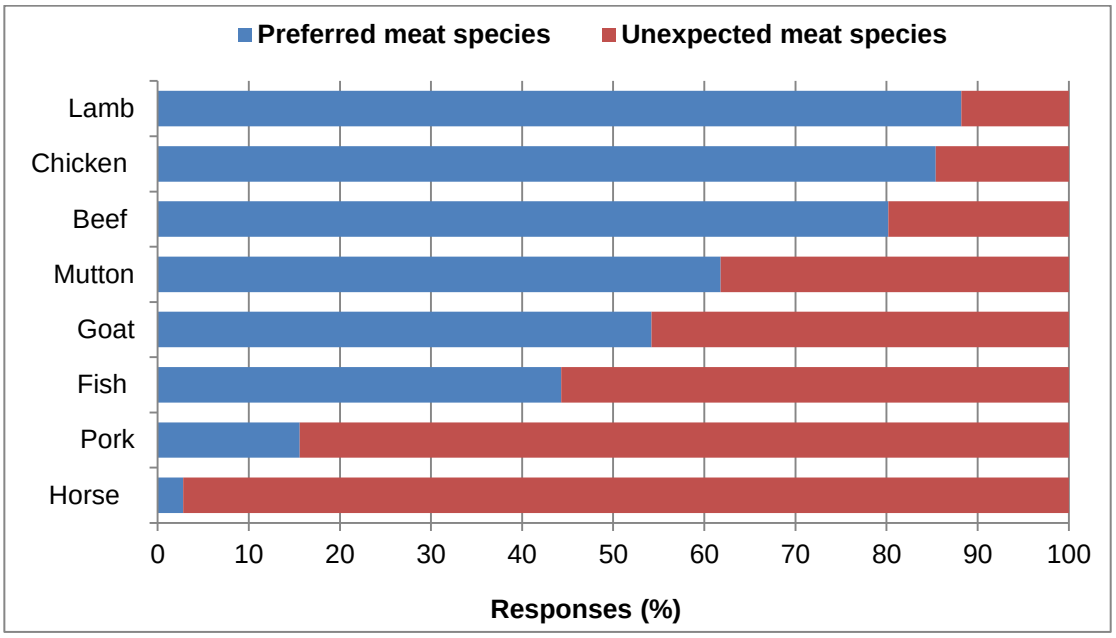


Figure 2.9: Preferred and unexpected meat species in KMP in the UK

Declaring the types of meat species in meat products is very important for consumers to make decisions, but a survey carried out across the UK by the FSA found evidence of substitution of lamb for cheaper meats such as beef and chicken. Samples (n=307) were from lamb dishes such as curries and KMP, sold from takeaway outlets. About 223 samples (73%) were fully compliant with food legislation, whereas 65 samples (21%) were positive for the presence of undeclared meat such as beef and chicken and 12 samples (4%) contained undeclared allergens including peanut and almond proteins, while 7 samples (2%) were non-compliant of the unauthorised use of additives. However, the level of undeclared meat species of 23 out of 65 were below 1%, which is more

likely an indication of poor handling rather than potential adulteration (FSA, 2015).

Cawthorn *et al.* (2013) reported the high incidence of other species and plant proteins (soya and gluten) substituted in several processed meat products in South Africa. Furthermore, a survey conducted in the UK by LACORS on doner kebab samples that were tested positive for pork even though they stated they were “halal”.

According to the Meat Products Regulations 2003, KMP supplied by manufacturers to sellers (kebab shops and takeaways) are subjected to the requirements of the Food Labelling Regulations 1996. For example, for a single meat species the percentage of meat must be included (e.g. lamb 40%), while for a mixture of meat species must also be declared (e.g., lamb 20%; beef 10% and chicken 10%) (LACORS, 2009).

Therefore, knowing the origin of meat species is very important. A negative statement has been asked of respondents in both countries (Question 20, Appendix 2.9) as follows; “knowing the origin of meat will not influence my choice to eat KMP”. Results shows that 33.5% of the UK respondents strongly disagreed with the statement compared to only 22.8% for respondents in KRI. In contrast, about 22% of respondents in KRI strongly agreed in comparison to only 9% in the UK (Table 2.14, and 2.15).

This can be summarized in that respondents in the UK were more concerned to know the origin of meat species than respondents in KRI. This could be because the horsemeat scandal had affected UK participants, and they demanded to know the origin of meat products (O'Mahony, 2013).

In regards to the meat content in KMP, the preferred average meat content in FG discussions was 72.5%. In this case, fourteen participants (n=20) preferred the meat content to be at least 70% (Table 2.8). In the WBS, four questions (Questions 12, 13, 14, and 15, Appendix 2.9) were designed to ask respondents to select their preferred acceptable meat content in KMP and the perceived meat content that the respondents believe is contained in KMP (shish and doner kebabs).

According to the results of the WBS in both countries, around 65-70% of the participants preferred the level of meat content to be at least 70%. Moreover, respondents in both WBS confirmed that the perceived meat content in shish and doner kebabs is less than their accepted levels in the UK (Figure 2.10, 2.12) and in KRI (Figure 2.11 and 2.13). Therefore, there was a highly significant ($p < 0.001$) difference between acceptable and perceived meat content in KMP in both countries (Appendix 2.11).

For example, around 65% of respondents accepted meat content of at least 70% or more in doner kebab, while about 40% of respondents preferred the acceptable levels to be in between 65% to 35%. In contrast, about 60% of respondents assumed that the actual meat content in doner kebabs is less than 70%, while the rest assumed it could be more than 70% (Figure 2.10 and 2.11).

Whereas in shish kebab, 70% of participants confirmed their acceptance of meat content to be at a level of 70% or more, while only 30% of respondents assumed the acceptable meat content to be in the range of 35-65%. Furthermore, about 50% of participants assumed that the actual meat content in shish kebab is 70% or more, which is more than in doner kebab (Figure 2.12 and 2.13).

Although no published papers were reported on the literature review regarding the minimum acceptable meat content in KMP, the meat content in similar processed meat product such as burgers should be from 62% to 67% depending on the types meat species used (FSA, 2003). A survey tested a total of 3174 samples included 403 samples of lamb kebab (doner and shish kebab) for species substitution by FSA during 2012/13. Lamb kebabs were among the most frequent samples that were not compliant with labelling descriptions where 104 out of 403 samples were tested positive for species substitution (FSA, 2013b).

In response to the question in the WBS as to whether respondents would eat KMP if the meat content was less than 65% in KMP (Question 20, Appendix 2.9), about 42% of respondents strongly disagreed; in contrast, 22% of the respondents in KRI and 36% in the UK agreed and strongly agreed to eat the product if the meat content was less than 65% (Table 2.14, 2.15). Although there were no such differences between respondents in both countries, Kurdish respondents seemed to require more meat content in KMP compared with the respondents in the UK. This can be linked to the earlier question that Kurdish participants tended to eat KMP more frequently.

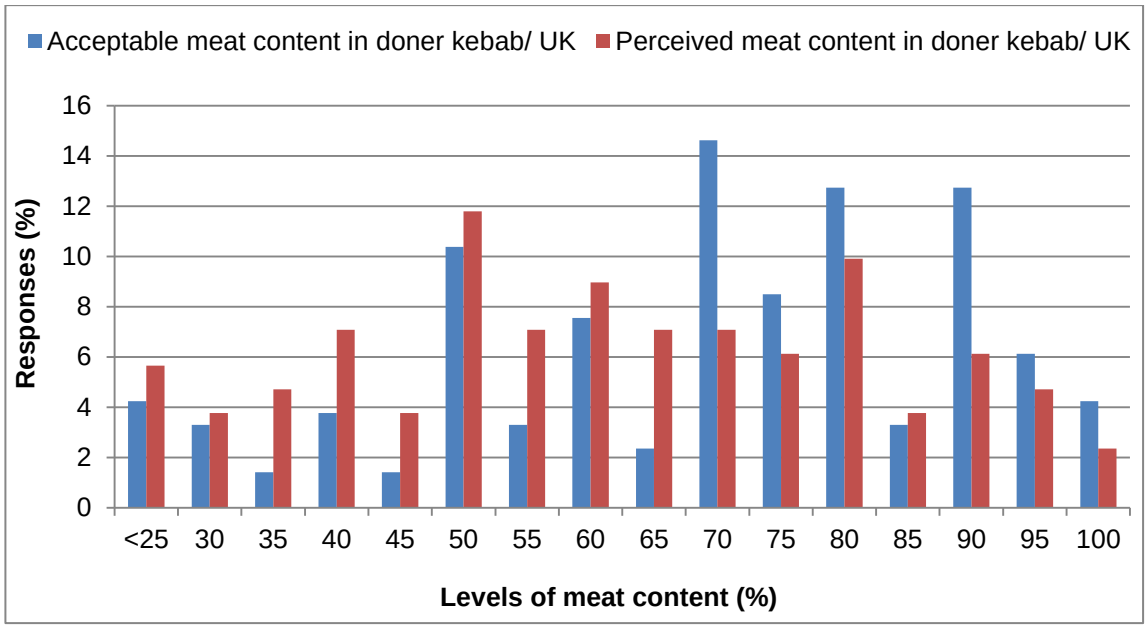


Figure 2.10: Acceptable and perceived meat content in doner kebab gathered by the participants in the UK

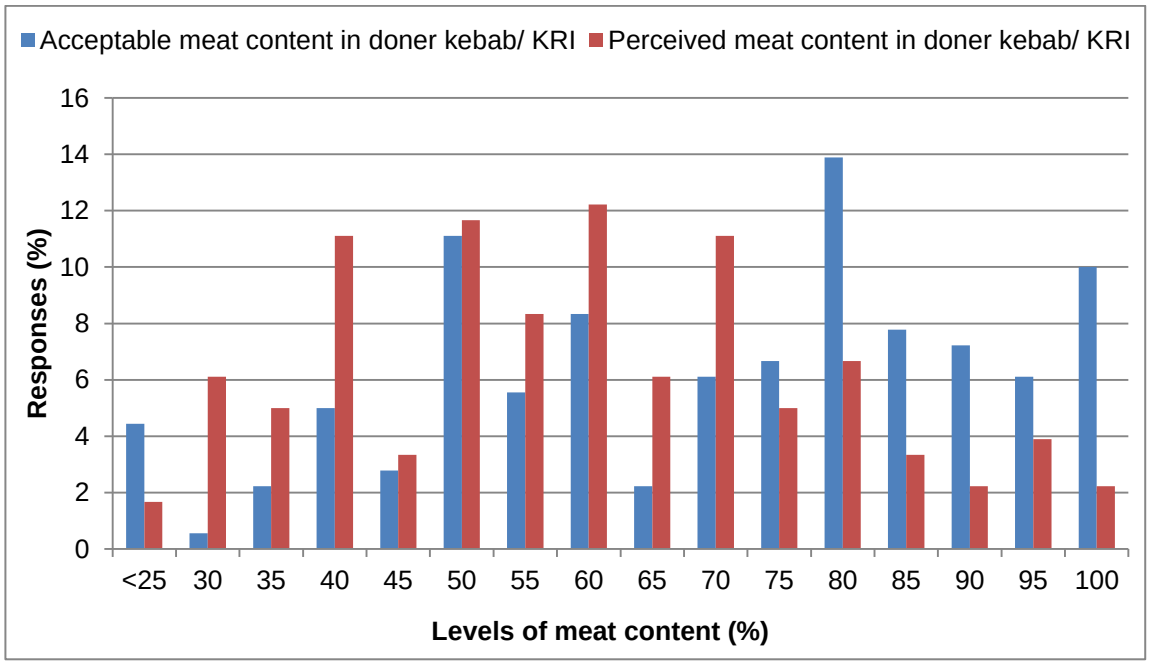


Figure 2.11: Acceptable and perceived meat content in doner kebab gathered by the participants in KRI

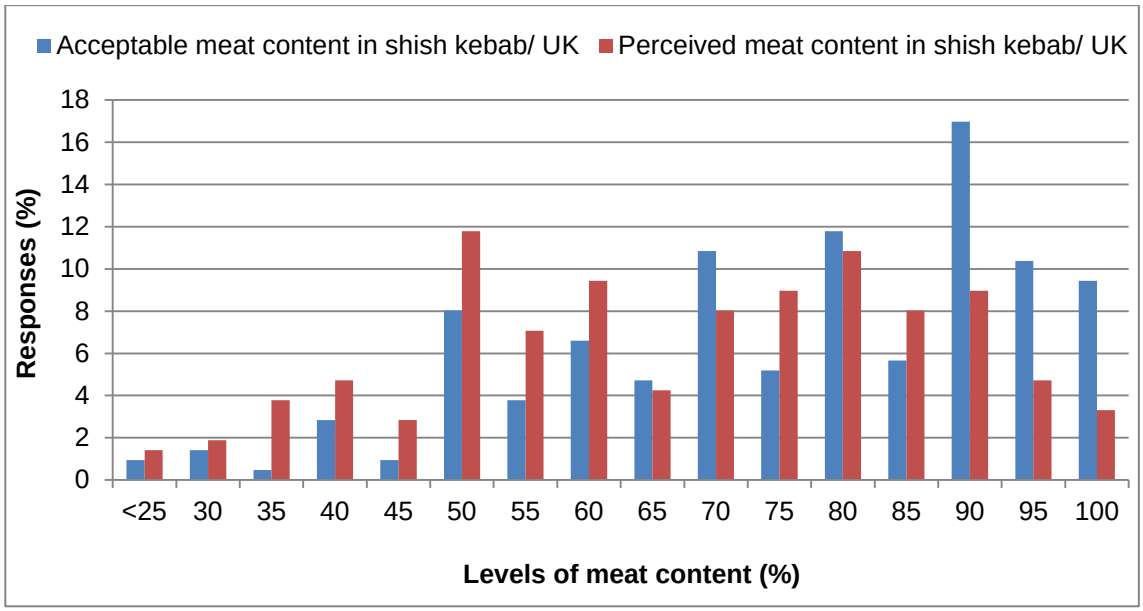


Figure 2.12: Acceptable and perceived meat content in shish kebab gathered by the participants in the UK

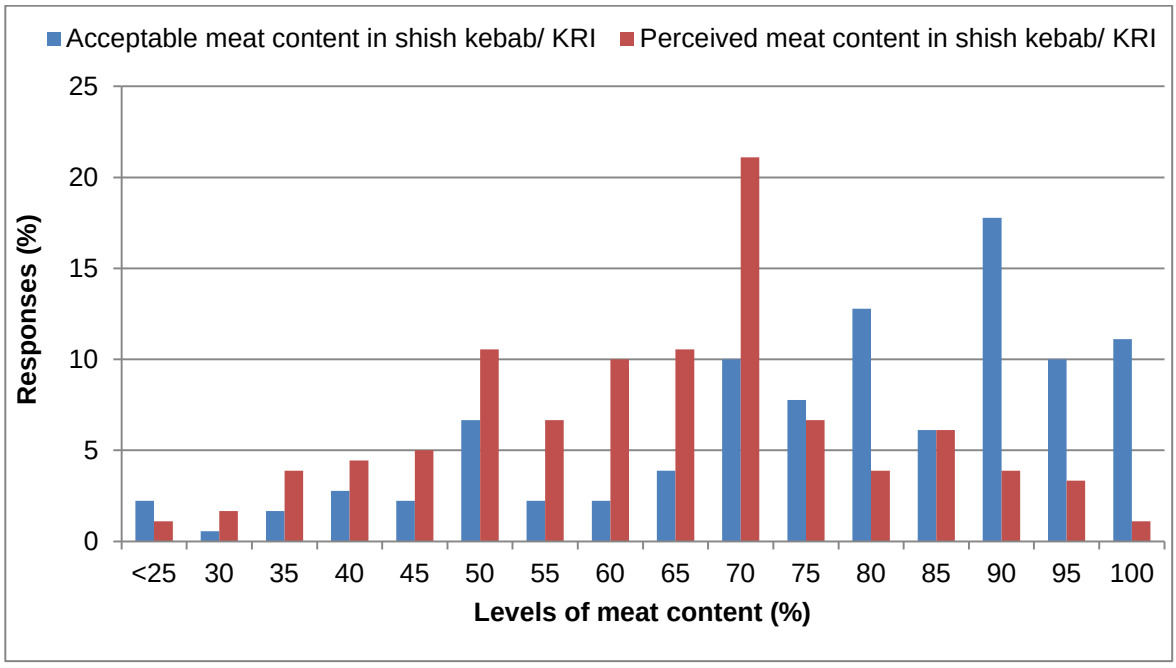


Figure 2.13: Acceptable and perceived meat content in shish kebab gathered by the participants in KRI

In addition to the meat species and the levels of meat content, it is important that other ingredients that are contained in KMP are declared. This is because often cheaper ingredients were added to KMP. For example, Everstine *et al.* (2013a) reported that seven in ten lamb kebabs sold in British takeaway restaurants were bulked up with cheaper meats. Therefore, respondents in the FG (Question 6, Appendix 2.5) and in WBS (Question 16, Appendix 2.9) approaches were questioned about their opinion of the additional ingredients that may be a part or a potential part of manufacturing KMP. For instance, the data presented in Table 2.8 was the list of ingredients that were proposed by participants in the FG to be a part of manufacturing KMP.

Additionally, a prepared list of ingredients was proposed for WBS participants and they were asked whether they should be used and in what proportions. The results of the Friedman test showed the average weight of each variable based on the rating scales for the respondents in the UK (Table 2.9). Bread, wheat (flour), and dietary fibres (e.g., inulin) were the most favoured ingredients, while alternative meat cut and MRM were the least acceptable ingredients to be used in manufacturing KMP in both countries (Figure 2.14, and 2.15). For instance, around 50% and 40% of participants in the UK responded that the MRM and alternative cut meats shall not be used respectively. In contrast, approximately 50% of the respondents were agreed that bread should be used in KMP, while this trend was about 40% with wheat (flour) in both countries. However, respondents in KRI tended to be slightly more in favour of additional dietary fibres (Figure 2.15) compared to their counterparts in the UK (Figure 2.14).

Table 2.8: Participants' preferences of meat species, meat content and other ingredients in KMP (FG, n=20)

participant Code	Preferred meat content	Preferred meat species	Ingredients preferred to be used in KMP
A1	70%	Chicken, lamb	Hot spices
A2	80%	Lamb	Less fat and salt
A3	60%	Chicken	Spices, tomato, sweet corn, beans
A4	30%	Lamb	Digestible flour, less fat
A5	50%	Chicken	Baked beans, sweet corns, onion
B1	50%	Lamb	Pitta bread, salad, spicy sauce
B2	100%	Lamb, beef	Only meat with less fat
B3	70%	Lamb	Tomato, sauces, and mayonnaise
B4	80%	Lamb	Salad, bread, garlic, celery
B5	80%	Lamb, chicken	Fresh bread fat and salad
B6	60%	Lamb	Salad, bread, garlic, chips
C1	90%	Lamb	Onion, garlic, spices
C2	80%	Lamb, chicken	Onion, vegetable and spices
C3	80%	Lamb	Spices, preservatives
C4	85%	Lamb	Onion, vegetables and spices
D1	85%	Lamb	Fat and spices
D2	70%	Lamb	Those to improve taste
D3	90%	Lamb	Parsley, onion, salt
D4	80%	Lamb	Spices and garlic
D5	60%	Lamb	Flour, antioxidants, onion

Table 2.9: Ranking of ingredients as to whether they should be used and in what proportions in KMP from respondants of the WBS in the UK (n=241).

Variables	Mean±SD	Mean rank
Bread	2.45±0.86	6.10
Wheat (flour)	2.33±0.86	5.80
Dietary fibre (e.g., inulin)	2.19±0.89	5.25
Wheat bulgur (cracked wheat)	2.11±0.83	5.08
Fat	2.06±0.83	5.00
Soybean ingredient	2.07±0.83	4.89
Starch	2.02±0.79	4.79
Alternative meat cuts	1.90±0.87	4.39
MRM	1.69±0.86	3.69

*Rating scales were 1=shall not be used, 2= acceptable in low proportion, 3= acceptable in medium proportion, and 4= acceptable in any proportion.

Previous studies showed the benefits of adding fibres such as soy protein and tomato pulp on chicken doner that improved cooking loss, colour and sensorial properties (Gök *et al.*, 2014). Moreover, the idea of adding dietary fibre such as inulin was also discussed in the FG (Question 7, Appendix 2.5). More than half of participants indicated that the main purposes of adding dietary fibre should be to improve the overall quality, especially lowering the fat content in KMP, though five participants did not agree with adding fibres (Box 2.3).

It seems that consumer reactions towards adding inulin is variable, and it is important to declare inulin content if it is intended to be added, as investigated in details regarding the technological benefits of inulin and the applied identification tool (Chapter 3).

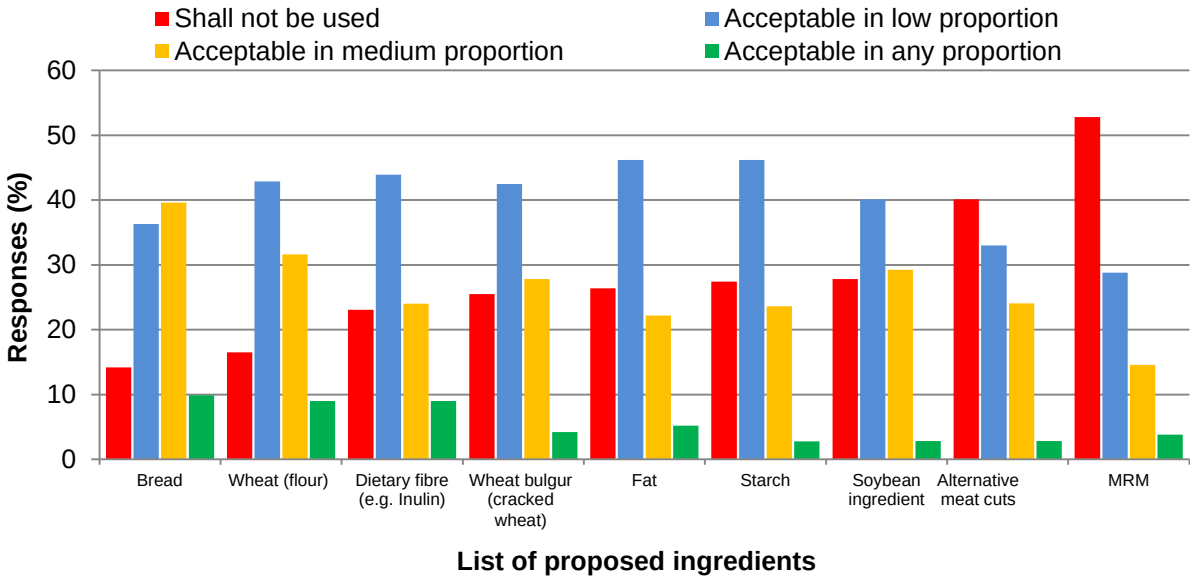


Figure 2.14: List of ingredients and participant responses in the WBS as to whether they should be used in manufacturing of KMP in the UK

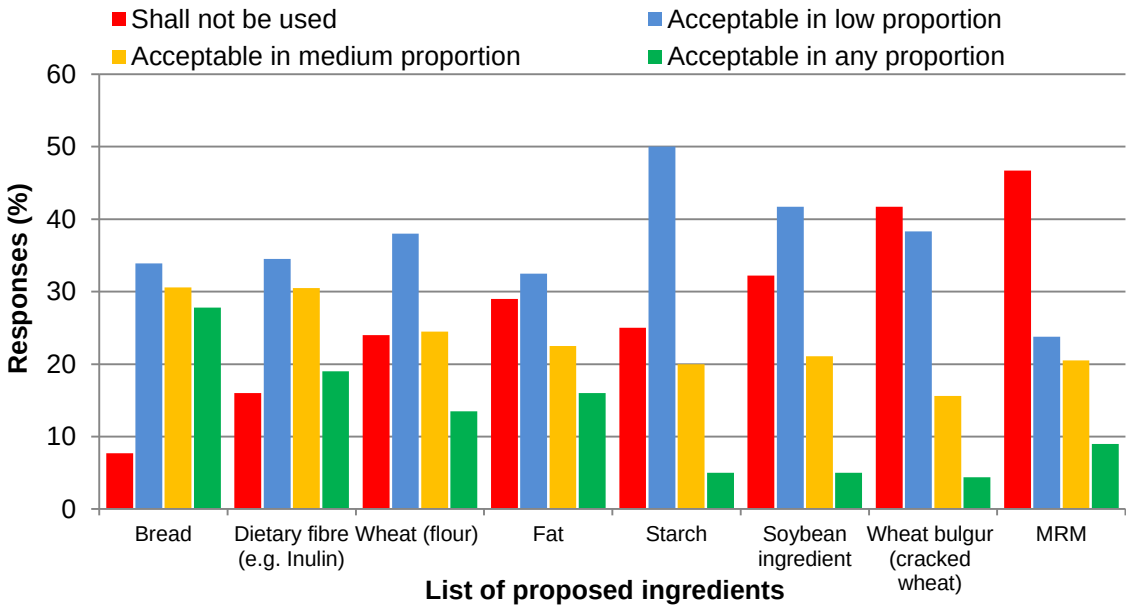


Figure 2.15: List of ingredients and participant responses in the WBS as to whether they should be used in manufacturing of KMP in KRI

Box 2.3: Typical comments about the idea of adding dietary fibre (inulin) in KMP discussed by FG

- "Does not agree with the idea of adding dietary fibres, feels it should be just meat" [A1 and A2]
- "Dietary fibres are very important; it can make the food more digestible" [A4]
- "Dietary fibres make meat products healthier, tastier, and reduce the fat content" [B5].
- "Dietary fibres are very important for health, but it should not reduce the quality of kebab" [B3]
- "Do not mind of adding dietary fibres, but does not want too much and it should not overtake the meat content" [C3]
- "Not a good idea to add dietary fibres, enough additives already" [D1]

Consumers feeling with several variables were also investigated in the WBS (Question 18, and 19, Appendix 2.9). This was in response to the concerns of a published survey by 76 individual councils in the UK reported by the LACORS coordinated food standard sampling programme (2009). According to the traffic light system, the obtained data in regard to content, total composition, and nutritional values of doner kebab indicated that 97%, 98% and 96% of the doner kebabs would be red for fat, saturated fat, and salt respectively. The survey found that each doner kebab as an average contains more than 1000 calories which is more than half of the Guideline Daily Amount (GDA) for a woman and 40% for a man. It also provides 89% of fat, 148% of saturated fats, and 98% for

salt of GDA for a woman and 66% of fat, 98% of saturated fats and 98% of salt for a man (LACORS, 2009).

First, respondents were asked for their feelings if an attribute or ingredient is not declared in KMP. The weighted average of the variables was listed from least disappointed to most disappointed according to the Friedman test (Table 2.10, and 2.11) in both countries. About 75% of respondents in the UK were strongly disappointed with the non-declaration of horse meat (Figure 2.16). Around 25% of respondents in both countries did not mind the non-declaration of vegetable ingredients, flour, starch and bread (Figure 2.16 and 2.17).

Although the provided information on halal products is voluntary according to the Food Labelling Regulation (1996), any such references must be accurate and not mislead the consumer. However, a survey conducted in the UK by LACORS on doner kebab samples that were stated as “halal” (literally means “permissible”) were tested positive for pork (LACORS, 2009). Therefore, the non-declaration of halal products was among the first two more disappointed statements in KMP in both countries.

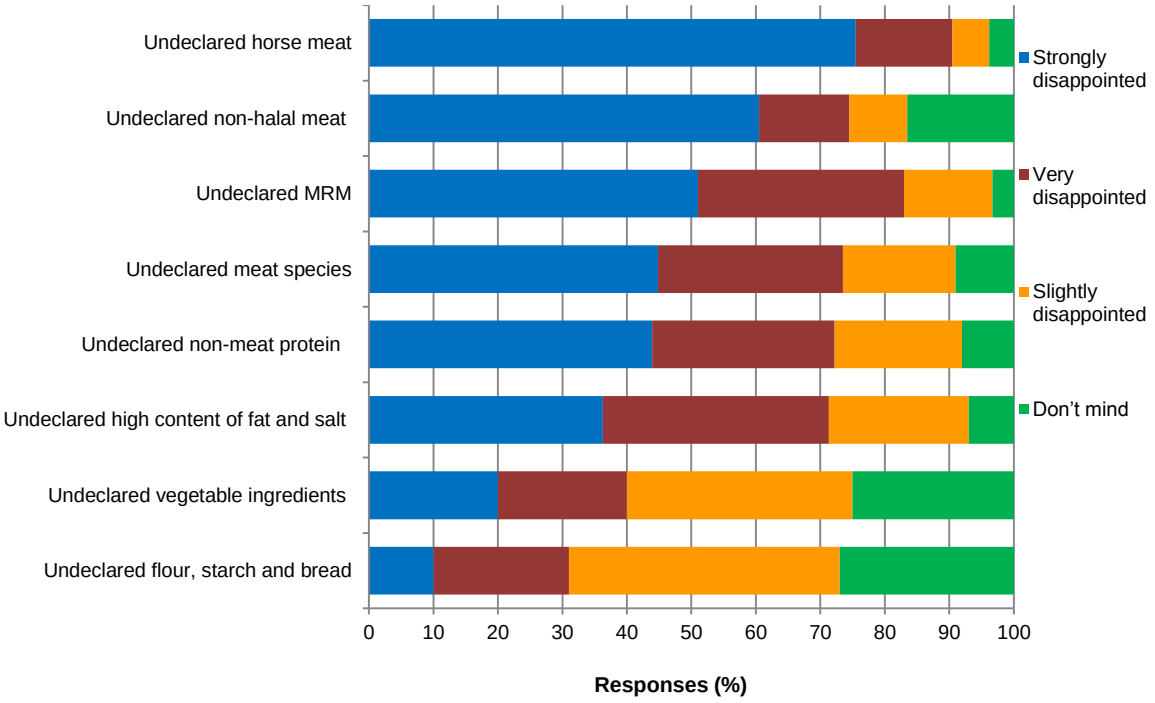


Figure 2.16: Participants' responses of the WBS regarding certain undeclared attributes in KMP from the UK (n=2401)

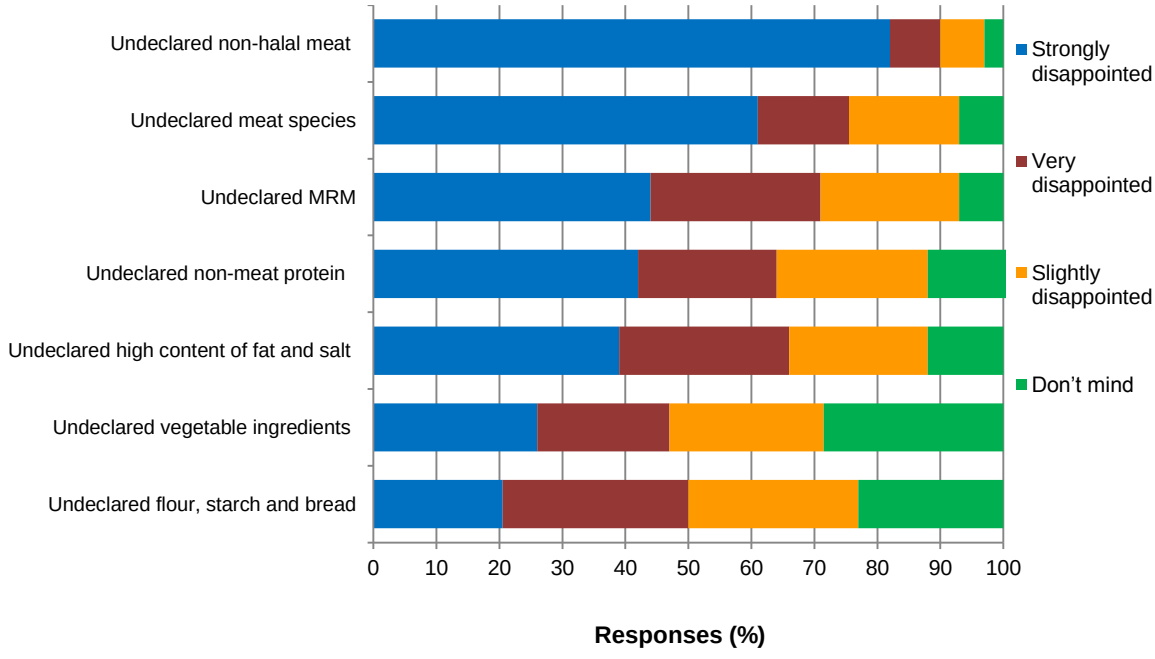


Figure 2.17: Participants' responses of the WBS in KRI (n=180) regarding certain undeclared attributes in KMP

Table 2.10: Participants' responses of the WBS regarding certain undeclared attributes in KMP in the UK (n=241)

Undeclared attributes in KMP	Mean±SD	Mean rank
Flour, starch and bread	2.86±0.93	6.26
Vegetable ingredients	2.64±1.07	5.82
High content of fat and salt	2.00±0.93	4.50
Non-meat protein	1.92±0.98	4.29
Meat species	1.91±0.99	4.27
Non-halal meat	1.82±1.15	3.96
MRM	1.69±0.83	3.83
Horsemeat	1.38±0.76	3.07

*Rating scales were 1=strongly disappointed, 2= very disappointed, 3= slightly disappointed, and 4= don't mind

Table 2.11: Participants' responses of the WBS regarding certain undeclared attributes in KMP in KRI (n=180)

Undeclared attributes in KMP	Mean±SD	Mean rank
Flour, starch and bread	2.52±1.06	4.95
Vegetable ingredients	2.55±1.16	4.91
High content of fat and salt	2.07±1.05	4.09
Non-meat protein	2.07±1.07	4.14
MRM	1.92±0.96	3.83
Meat species	1.71±1.01	3.42
Non-halal meat	1.30±0.72	2.68

**Rating scales were 1=strongly disappointed, 2= very disappointed, 3= slightly disappointed, and 4= don't mind

It is always important to consider consumer concerns regarding meat and meat products (Ergönül, 2013). Therefore, respondents were asked for their concerns regarding several attributes in WBS (Question 19, Appendix 2.9). Respondents' concerns in UK were mostly on the hygienic condition, horsemeat presence, and types of meat species (Figure 2.18 and Table 2.12). In contrast, price and calories of meal were least concerned.

Similar results were also obtained with participants in KRI, where hygienic condition was the most concerning followed by the types of meat species and the amount of fat and salt in KMP, and the least concerning variables were price and calories of meal (Figure 2.19). Although calorie levels of KMP was among the variables causing least concern, equally around 55% of the respondents in both WBS expressed their concerns (very concerned and somehow concerned) over the high calorie content on KMP (Figure 2.18, and 2.19).

Furthermore, a statement has been asked of respondents in the WBS if the kebab meal contents (soy protein or gluten) could produce potential allergies and should therefore be declared. Although there were no such significant differences between respondents in the UK and in KRI, respondents in the UK tended to more agree with the statement compared to the respondents in KRI (Table 2.14, and 2.15).

Labelling and nutritional value of KMP was also questioned during the FG sessions (Question 8 and 9) and in WBS. Although, many participants in FG were not satisfied with the nutritional values of KMP and assumed by some as unhealthy food, they want to keep eating the product but not frequently. Participants had different point of view on labelling of KMP on catering services (Box 2.4). For example, some participants preferred KMP to be labelled with the

types of meat species, the levels of meat content and other ingredients. Furthermore, a quarter of participants (25%) were willing to pay premium prices for proper labelling. However, a few participants stated that labelling of KMP is not a legal requirement and they were not willing to pay premium prices for labelling.

Box 2.4: Comments by the participants about labelling

- "Only sometimes trusts what the KMP seller says, important to know what you are eating, will pay more for labels" [A3]
- "Wants to know species, nutritional information and source of meat, will avoid a restaurant if there is no ingredient list" [B6]
- "Should be labelled as you do not always know what is inside the meat, will pay more for the proper labelling, has asked shop manager but they did not know the ingredients" [B5]
- "Should be labelled to discover origin of the food, should not have to pay more for labels, don't think it is a legal requirement" [C3]
- "Trust restaurants, would not ask for recipe, not expecting labels from restaurant" [C4]
- "Is a good idea, cannot trust labels as the factory could add anything and not mention it, would not pay more for labels, never thought about kebab labelling until now" [D1]
- "Would like a label saying calorie content but never asked to see a label and does not feel that they would know, would not pay more for labels" [D2]
- "Doesn't care, if you worry about the contents you would not enjoy it" [D3]

In addition, respondents in the WBS were also questioned regarding the labelling of KMP (Question 20, Appendix 2.9), according to the following three statements as presented in Table 2.14 and 2.15 for the respondents in both countries. In the first statement; “I will pay more if the KMP are well labelled”, interestingly, respondents in KRI were slightly more willing to pay premium prices for better labelling compared to those in the UK. Equally, about 54% of both respondents disagreed and strongly disagreed with this statement. “Having labelling on KMP will not affect my decision when buying”. A similar trend was also observed for the statement “I would prefer KMP to be labelled and with all ingredients”, in that there was no such differences between respondents in both countries.

According to the traceability assurance schemes, all parties of the food supply chain should follow the regulations in order to maintain and restore consumer confidence (Watson, 2000). Therefore, the impact of the recent horsemeat scandal in Europe was questioned in the WBS to find out whether it has any influence on purchasing decision on KMP. This question was in response to a drop in consumers’ confidence when purchasing processed meat products, as published in several studies.

In the present study the impact of the horsemeat incident on the respondents’ perception was as follows. About 75% of respondents in the UK were strongly disappointed with the non-declaration of horse meat (Figure 2.16). Furthermore, more than 65% of participants were very concerned about the presence of horsemeat on KMP (Figure 2.18), and it was the second most concerning variable for the respondents in the UK (Table 2.12). Moreover, participants were also asked regarding changes in eating habit as a result of the horsemeat

incident. Results showed that about 40% of the participants in the UK agreed that they eat fewer processed meat products including KMP, while about 38% of the participants disagreed or strongly disagreed with the impact of the horsemeat scandal (Table 2.15).

A survey conducted by FSAI of more than 1000 adults showed that almost all participants (98%) were aware of the horsemeat scandal and about 51% had significantly changed their purchasing habits (Robinson, 2013). Another survey found that sales of beef products in the UK decreased by 3% over the year of the scandal. This was most significant in frozen burgers and frozen ready meals in supermarkets. As a result, more than 30% of the adults changed the way they buy and choose the food, while 10% tended to eat fewer processed meat products (Food Processing, 2014). Reducing consumers' confidence not only affected the processed meat products in the UK, but also significantly damaged the meat sector in the EU with sharp decline in the ready meals (O'Mahony, 2013).

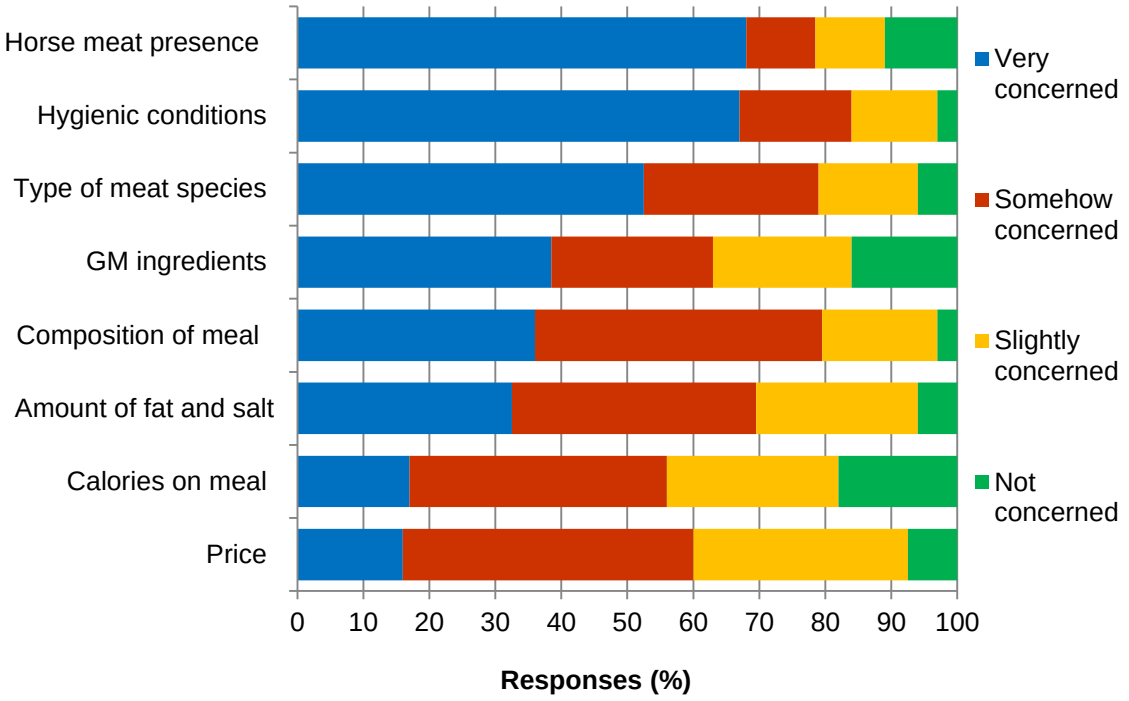


Figure 2.18: Participant concerns of several attributes of KMP from UK WBS

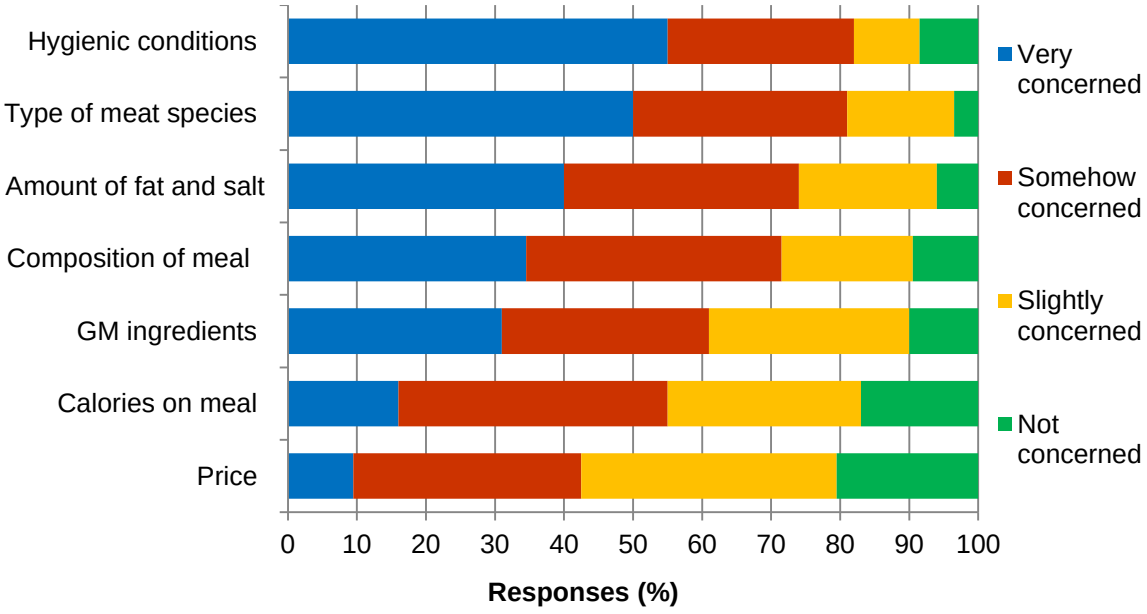


Figure 2.19: Participants' concerns on several attributes of KMP from WBS in KRI (n=180)

Table 2.12: Respondents' concerns on several attributes of KMP in the UK (n=241)

Variables	Degree of concerns Mean±SD	Mean rank
Hygienic conditions	3.47±0.845	5.60
Horsemeat presence	3.35±1.058	5.33
Type of meat species	3.25±0.928	5.07
Ingredients/ composition of meal	3.13±0.798	4.69
Amount of fat and salt	2.96±0.904	4.27
GM ingredients	2.86±1.105	4.15
Price	2.68±0.827	3.54
Calories on meal	2.55±0.975	3.33

*Rating scales were 1=not concerned, 2= slightly concerned, 3= somehow concerned, and 4= very concerned

Table 2.13: Respondents' concerns on several attributes of KMP in KRI (n=180)

Variables	Degree of concerns (Mean±SD)	Mean rank
Hygienic conditions	3.29±0.95	4.83
Type of meat species	3.28±0.85	4.77
Amount of fat and salt	3.09±0.93	4.38
Ingredients/ composition of meal	2.97±0.96	4.10
GM ingredients	2.82±0.99	3.86
Calories on meal	2.54±0.95	3.26
Price	2.31±0.91	2.80

*Rating scales were 1=not concerned, 2= slightly concerned, 3= somehow concerned, and 4= very concerned

Table 2.14: List of statements questioned respondents in the UK about their opinion on KMP in the WBS based on the degree of agreement and disagreement (n=241)

Statements	Responses (%)					Average weight (mean rank)
	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly Agree	
If kebab meal contains (soy protein or gluten) and could produce potential allergies , it must therefore be declared	6.6	5.2	16.5	34	37.7	9.48
If the meat content was under 65%, I would still eat kebab	13.7	29.7	24.5	25.9	6.2	6.33
I never worry about the quality of my kebab meal	39.6	35.9	13.2	9.4	1.9	3.93
Information about kebabs influence whether or not I buy KMP	4.3	13.2	23.1	38.2	21.2	8.45
I do not trust any meat that is minced regardless where I buy it	7.5	31.1	27.4	23.1	10.9	6.68
I don't think kebabs are healthy	7.1	16	35.8	27.4	13.7	7.58
Having labelling on KMP will not affect my decisions when buying	16.5	38.2	17.5	22.2	5.6	5.83
I am always very satisfied with KMP	7.6	26	41	20.8	4.7	6.54
I would prefer KMP to be labelled and with all ingredients	1.9	5.2	16.5	34.9	41.5	9.95
Knowing the origin of meat will not influence my choice to eat KMP	33.5	28.3	16	13.2	9	5.05
I will pay more if the KMP well labelled	2.4	10.4	30	36.4	20.8	8.52
I would like to avoid eating KMP	15.1	30.2	33.5	14.6	6.6	5.81
Due to recent incident with horse meats, I eat less processed meat products including KMP	13.2	24.5	23.6	22.7	16	6.85

Table 2.15: List of statements questioned respondents in KRI about their opinion on KMP in the WBS based on the degree of agreement and disagreement (n=180)

Statements	Responses (%)					Average weight (mean rank)
	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly Agree	
If kebab meal contains (soy protein or gluten) and could produce potential allergies, it must be declared	8.9	11.1	23.3	31.7	25	7.89
If the meat content was under 65%, I would still eat kebab	18.9	23.2	35.6	15	7.2	6.33
I never worry about the quality of my kebab meal	29.4	27.2	26.7	10	6.7	5.02
Information about kebabs influence whether or not I buy KMP	5	12.8	19.4	35.6	27.2	8.3
I do not trust any meat that is minced regardless where I buy it	23.2	18.9	17.8	21.1	18.9	6.34
I don't think kebabs are healthy	11.7	15.6	25.5	30	17.2	7.23
Having labelling on KMP will not affect my decisions when buying	27.2	26.1	25.6	18.3	2.8	5.15
I am always very satisfied with KMP	17.8	22.8	33.3	20	6.1	5.93
I would prefer KMP to be labelled and with all ingredients	2.2	2.2	15.6	30.6	49.4	9.97
Knowing the origin of meat will not influence my choice to eat KMP	22.8	11.7	24	20	21.5	5.05
I will pay more if the KMP well labelled	4.4	10	22.2	31.2	32.2	8.61
I would like to avoid eating KMP	10	19.4	31.7	18.9	20	6.94
Due to recent incident with horse meats, I eat less processed meat products including KMP	25.6	10.6	15.5	19.4	28.9	7.21

2.3.4 Hygienic conditions and safety incidents of kebab products

In the last question of FG, safety or hygienic conditions of kebab shops were discussed. More than half of the participants in all four groups raised their concerns and were not satisfied with hygienic conditions of KMP especially at catering services (Box 2.5).

Box 2 5: Typical comments by the participants of the FG on the hygienic conditions of fast-food establishments

- "Doesn't think it is very hygienic, needs more government intervention" [A1]
- "Not satisfied, restaurants need more inspection and government is responsible for safety inspection" [A2]
- "Friends have had negative experiences with kebab hygiene (e.g., food poisoning), staff should wear hats and gloves" [A5]
- "Some are very unhygienic, has been served raw meat and blood seen in kebabs, more regulations and inspection needed" [B3]
- "Not very clean, instruments should be cleaned more often; feels staff might not meet regulations and should wear more hygienic clothes" [B4]
- "Happy with safety of restaurants, feels it should be more regulated by government" [C2]
- "No negative experience, feels it is regulated well by the government" [C3]
- "Items found in kebabs i.e. a pin, you can't see what is out back- could be hygienic issues and the customers don't know about it" [D1]
- "Safety measurements in the UK are very good, people follow hygienic requirements and equipments are cleaned" [D3]

They mentioned problems of cleanliness at catering services especially in kebab shops, and concerns were related mostly with food poisoning. Many participants in all four groups expressed distrust about the level of cleanliness and sanitation in kebab shop and restaurants. Worries were expressed about handling, cooking, used instruments and staff personal hygiene. It is well known that consumer distrust could be raised as a consequence of contamination of foods and inadequate hygiene regimes in food outlets (Kjaernes *et al.*, 2007).

Generally, smaller premises such as take-away catering have been highlighted as an area for concern with regard to ready-to-eat food and food safety risks in the UK (Little *et al.*, 2003). Furthermore, a UK FSA survey on consumer attitudes indicated that hygiene standards in take-away and fast food premises are a key area of concern for consumers (FSA, 2004).

Generally, the quality and safety of kebab meat depends on several factors such as the quality of raw materials, efficiency of cooking process, sanitation of facility of kebab making and personal hygiene (Todd *et al.*, 1986).

Therefore, it is important to increase the awareness through improved training, such as HACCP principles, of all food handlers and managers that may lead to an improvement in hygienic practices in smaller premises (Little *et al.*, 2003).

Regarding the requirements for hygienic inspection in the present study, a number of participants agreed that more regular inspections are needed. However, one third (35%) of participants especially in group 3 and group 4 were satisfied and stated that there are clear enough regulations in the UK for safety inspection. According to a focus group conducted at Reading University, most

participants agreed that the government need to play a bigger role in regulating food manufacturers and force them to produce healthy food (Chambers *et al.*, 2007).

The sociodemographic characteristics of respondents have been included in the analysis, since they are frequently recognised as important factors for better understanding how consumer demand would be established and how to identify respondents' characteristic orientation. Therefore, the findings from this study can be outlined according to the sociodemographic factors as follows:

Cultural orientations:

Respondents classed as Middle Eastern included the Kurdish, who tended to eat and spend money more frequently on KMP compared to British/ European and those from other cultural background. Furthermore, participants in ME including Kurdish were more trusting of KMP. Although beef burgers and sausages were the least trusted fast foods by the majority of respondents, British/ European respondents do consume and trust beef burgers and sausages more than the other three cultures. Chicken meat was preferred by other cultural groups more than by ME, British/EU and Kurdish. Respondent in the UK were more concerned with knowing the origin of meat species than in KRI and were required less meat content in KMP compared to Kurdish.

Moreover, UK respondents expressed their worry more than participants in KRI regarding the quality of KMP. Surprisingly, respondents in KRI were slightly more willing to pay more if the KMP are well labelled compared to respondents

in the UK. They were also slightly more in favour of adding dietary fibre such as inulin. Therefore, it can be concluded that Kurdish and British participants do have different perceptions of eating habits.

Period spent in the UK:

Generally, respondents who lived in the UK for less than 6 months had eaten KMP more frequently compared to others, and these respondents were assumed to be in the category of ME or Kurdish where they eat KMP more frequently compared to those in the UK. Instead, participants who were in the UK for more than 5 years (most probably within British/ European) had significantly more trust on sausages and they had eaten beef burgers significantly more frequently than those who lived in the UK less than 5 years.

Gender:

Although various sociodemographic factors were associated with the frequency of eating KMP, gender has a particularly strong influence on meat consumption. Generally, male respondents tended to eat KMP significantly more frequently than females. In addition, men were more likely to trust shish kebab than women and had eaten significantly more shish kebab than women. On the contrary, women in KRI had more trust in pizza, while UK females had eaten beef burgers significantly more than UK males. Furthermore, women had more awareness of labelling and willingness to pay premium prices for better labelling.

Age groups:

In general, respondents aged less than 36 years old in both countries ate KMP with significantly more frequency than participants aged over 36 years old. In contrast, respondents over 41 years old in both countries had more trust in sausages compared to younger generation. In comparison, all participants in KRI aged over 41 years old had no trust at all in sausages. Education level and Employment status had less impact on the overall variables, and this could be due to the less variability among these two characteristics (Table 2.2).

2.4 Conclusion

The findings from FG and WBS provide a clear background on consumers' attitudes and trust towards KMP. For example, the three most important quality attributes behind choosing KMP over other fast foods were: taste, freshness, and late open availability of kebab shops, while the web-based surveys highlighted "taste and safety of meal" as the most important attributes in both countries.

In general participants in the FG were morelikely to prefer and trust shish kebab compared to doner kebab, whil beef burgers and sausages were the least trusted products in both countries. Participants in both protocols indicated their preferences toward lamb meat followed by chicken meat and they preferred KMP of a single meat species rather than mixed meat. In addition, they selected an acceptable meat content of at least 70% in KMP.

However, respondents in both countries stated that the percieved meat content in shish and doner kebabs was lower than what they see as their acceptable levels. Addition of others ingredients such as dietary fibres (inulin) was seen as positive by half of repondents if used in KMP, whereas MRM and alternative meat cuts were considered by respondents not to be used in KMP.

The horsemeat scandal had slightly changed respondents' perception of purchasing processed meat products including KMP. There was some doubt about the labelling requirement by catering services, and there was a general trend for providing more information on KMP. There were cultural differences between participant's perceptions of both countries and among investigated cultural groups in the UK, and even between Kurdish respondents themselves in both countries. The sociodemographic characteristics of respondents have helped to better understand how consumer demand would be established and how to identify respondents' characteristic orientation.

The findings of this study are applicable to stakeholders in the food chain in order to identify exactly what questions and concerns consumers had regarding processed kebab composition, labelling, safety, and how consumer confidence could be restored. The results of this study suggest that consumer research study should focus on bringing consumers' food choices and trust in line with nutritional knowledge and should include efforts to help consumers' understanding and belief in the importance of personal food choices in determining health status.

This study also suggests that it would be better for catering establishments to provide more information on KMP, especially the declaration of meat content and meat species used. For instance, it should be declared whether a KMP is

made of a single meat species (lamb doner kebab) or from a mixed meat species such as lamb and beef doner kebab, compared to unspecified doner kebab. Furthermore, information should be accurate regarding the level of each meat species.

The key identified issues in this study indicated that respondents were in doubt regarding the content of KMP, especially doner kebab, and in particular species identity, the levels of meat content, the source of meat origin and their reaction towards additional ingredients such as inulin.

These key issues were selected as research questions to be further investigated in the followed chapters. For example, the consumer reaction towards additional of non-meat ingredients (inulin) as a fat replacer in KMP were examined and an analytical tool to identify the addition of inulin for labelling purposes was applied (Chapter 3). The second problem that consumers were most concerned was the species identity and the levels of meat content. However, instead of applying some analytical tools to identify meat species and origin, the next two chapters were focused on the fish species and the production method of fish (wild and farmed fish).

This is because the consumption of fish in KRI and in worldwide has increased over recent decades. Furthermore, due to the lack of labelling enforcement and traceability in place, consumers are concerned about the identity of fish species and the origin of production methods (wild and farmed fish) from fraudulent practices.

For this reason, DNA-barcoding was used to evaluate the potential of mislabelling of fish species in KRI (Chapter 4). However, even ensuring species

identity and avoiding species substitution, the consumers' concern may still exist regarding the origin source of identified species. Therefore, in Chapter 5, the potential of NIRS was examined to predict the chemical and minerals composition and discriminate between wild and farmed common carp origin.

Some other identified issues, such as respondents' concern to the levels of each meat species in a mixed meat product, could be an opportunity for fraud. Detection tools such PCR real-time and immunoassays were mostly used. However, due to time and expense limitations, these issues were added to future work list for further research.

Chapter 3

The use of oligosaccharide in meat products- investigating eating quality characteristics, consumers' acceptability and inulin determination

3.1 Introduction

Consumers are increasingly aware of diet related health problems, and therefore demanding safe and health promoting foods (Puupponen-Pimiä *et al.*, 2002). Improvements in the quality image of meat products are needed to meet consumer satisfaction (López-López *et al.*, 2010). Meat and meat products are considered to be of high nutritional and biological value including protein, valuable amounts of fatty acids, vitamins, minerals and other bioactive compounds (Olmedilla-Alonso *et al.*, 2013).

However, in spite of all the positive attributes, meats are deficient in essential dietary fibre (Papadima and Bloukas, 1999, Mehta *et al.*, 2015). Meat products are also recognized for having high fat content (Papadima and Bloukas, 1999, Biesalski, 2005, Tomaschunas *et al.*, 2013), especially those products from takeaway or fast food outlets (Papadima and Bloukas, 1999, Jaworowska *et al.*, 2013), including sausages, burgers, pork pies, and kebabs, which account for almost half of all meat consumed in developed countries (Kearney, 2010).

Doner kebab as a fast food is a traditional Middle Eastern meat product (Kayisoglu *et al.*, 2003, Gonulalan *et al.*, 2004, Kayaardi *et al.*, 2006, Kilic, 2009). The product is popular in restaurants and fast-food outlets in Middle East countries, Europe, Canada, USA, and the UK, and it contains high fat levels ranging between 20 and 40% (Kilic, 2009). Fats in meats play an important role in consumer acceptability and quality attributes such as texture, flavour, tenderness, juiciness and appearance (Zhang *et al.*, 2010, Rodriguez Furlán *et al.*, 2014).

However, epidemiological research has demonstrated the relationship between excessive consumption of diets rich in fats and the emergence of chronic diseases (Käferstein and Clugston, 1995, Micha *et al.*, 2010, Bhat and Bhat, 2011). These increasing concerns have driven the food industry to develop new formulations or modify traditional products to make them healthier (Garcıa *et al.*, 2002).

Therefore, the reduction or removal of fat from meat products is desirable, but challenging (Tomaschunas *et al.*, 2013, Keenan *et al.*, 2014), as there are many problems concerning their acceptance (Sandrou and Arvanitoyannis, 2000, Tomaschunas *et al.*, 2013), including the difficulties in maintaining attributes such as appearance, flavour, and texture (Tomaschunas *et al.*, 2013). As a result of reducing fat, the products become firmer, more rubbery, less juicy, darker in colour and less acceptable (Keeton, 1994, Mallika *et al.*, 2009).

Therefore, fat reduction in food formulations should be compensated with other ingredients that would have similar functional roles in maintaining sensory qualities (Devereux *et al.*, 2003). The use of non-meat ingredients can contribute to achieving desirable texture and enhance water-holding ability (Keeton, 1994, Colmenero, 1996). Several non-digestible carbohydrates and fibres have been used as fat replacement in foods (Archer *et al.*, 2004, Talukder, 2015) which can improve cooking yield, reduce formulation cost, enhance texture (Keeton, 1994, Colmenero, 1996), as well as reduce fat content, and subsequently lowering calories and cholesterol (Elleuch *et al.*, 2011). Inulin, a soluble dietary fibre, is often added to food products (Mendoza *et al.*, 2001, Selgas *et al.*, 2005) such as meat products at levels of 2-10 (w/w) (Franck, 2002). It has been approved for use in meat products according to the Food

Standard and Labelling Policy Book of the USDA- Food Safety and Inspection Service (FSIS) (Tarté, 2009). It is amongst the most studied and well established prebiotics (Gibson, 2004). It has been used as versatile ingredient in processed functional foods as fat replacer or as fibre supplement (Roberfroid, 2007). In addition, inulin has the ability to form stable gel networks which can be used to mimic some textural properties of fat when inulin is added to low- fat meat products (Bodner and Sieg, 2009). Incorporation of this ingredient in frequently consumed foods such as meats could help to overcome the fibre deficit in target populations (Ang and Miller, 1991), as the levels of dietary fibre intake for the general public around the world are below the recommendations (Cho, 2009).

Chicory and Jerusalem artichoke (JA) are two natural sources used for the industrial production of inulin (Lingyun *et al.*, 2007). Adding inulin from chicory into the processed meat products was found to be positive for improving quality and stability. There have been several attempts at introducing different types of fibres as fat replacers including commercial inulin from chicory root (Mendoza *et al.*, 2001, Devereux *et al.*, 2003, Selgas *et al.*, 2005, Luisa García *et al.*, 2006, Flaczyk *et al.*, 2009, Cegielka and Tambor, 2012, Keenan *et al.*, 2014) and in combination with other fibres (Archer *et al.*, 2004, Nowak *et al.*, 2007, Menegas, 2013, Tomaschunas *et al.*, 2013, Rodriguez Furlán *et al.*, 2014).

The use of inulin from JA in meat products is rare, but Gedrovica and Karklina (2013) confirmed an improvement on physical and textural properties of meatballs using inulin from JA as replacement for white bread. However, the challenge for both the scientific community and the food industries is to provide assurance to the consumers that these new food products are not just fraud

opportunities for profits that may mislead consumers, but to portray it as a genuine attempt toward better healthier food (Roberfroid, 1999). For example, inulin has become the target of adulteration for economic gains. The fraud involved the addition of low-priced sweetening products such as glucan prepared to have a similar profile to inulin (Wang *et al.*, 2010). Chicory was also adulterated in ancient times with roasted carrots and turnips (Shears, 2010). Furthermore, overconsumption of inulin could pose negative effects including signs of intolerance and a symptom of abdominal pain with the intakes above 20-30 g/day (Carabin and Flamm, 1999). Therefore, for labelling and control purposes of inulin in foods, several methods have been developed including enzymatic assay (Quemener *et al.*, 1994, McCleary *et al.*, 2000, Steegmans *et al.*, 2004).

The reduction of consumers' confidence when purchasing processed meat products in recent years was associated with the fraud incidents involving meat products in Europe (Walker *et al.*, 2013), also affecting confidence in doner kebab products. These products are candidates to be reformulated with functional ingredients such as dietary fibre (Selgas *et al*., 2005).

With these issues in mind, the objectives of this study were:

1- To investigate the consumer acceptability of the addition of JA flour and CI as new ingredients for replacing fat in prepared doner kebab.

2- To assess the effects of replacing of doner kebab fat with JA flour and CI, and the difference of the degree of chopping on the cooking characteristics, quality attributes, physicochemical and microstructure.

3- To identify and quantify the inulin inclusion on the JA flour, CI, and meat product for labelling purposes.

3.2 Materials and methods

3.2.1 Preparation of flour from Jerusalem artichoke tubers

The first source of inulin was prepared as flour from fresh tubers of JA (*Helianthus tuberosus*), which were harvested in France in October 2013 and obtained from a local market in Plymouth City Centre. The JA tubers were cleaned and washed with tap water to remove dust and debris. The cleaned tubers were peeled and sliced into small pieces (Laurenzo *et al.*, 1999), and dried at 50 °C for 48h, then ground to a powder (FOSS, Knifetec^{TM} 1095, Warrington, UK) (Yamazaki *et al.*, 1989). The powder was sieved using a shaking sieve to pass through a 0.250 mm mesh (Figure 3.1).

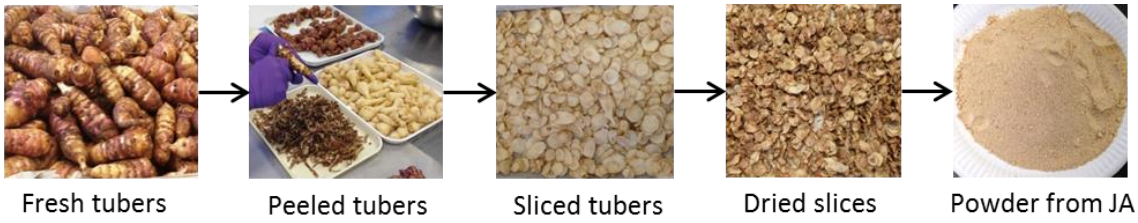


Figure 3.1: The processing steps for making JA flour from JA tubers, and variation of shape and colour of the material at intermediate stages

The prepared dried samples were packed and sealed in polyethylene bags and stored at room temperature, in a dry container, to avoid moisture absorption for further analysis (Modler *et al.*, 1993, Lingyun *et al.*, 2007). The prepared powders resembled flour, and could be potentially used as a starting material for commercial production of fructan. Generally flour from JA contains about 60-80% of a mixture of fructan and fructose that could be added into food products (Yamazaki *et al.*, 1989).

The second source of inulin was Frutafit® TEX (Sensus, Roosendaal, Netherlands) with long chain saccharide (Chiavaro *et al.*, 2007) derived from chicory roots and containing 96.7% inulin (Table 3.1), used and labelled as “commercial inulin” (CI).

The reasons for choosing JA in addition to the commercial inulin were due to its availability in KRI for further research, compared to inulin that is not available in KRI and need to be imported. JA is also easy to extract with a simple protocol (Figure 3.1) and its applications in food stuffs are rarely investigated in KRI.

In contrast, the commercial inulin is a natural powdered food ingredient based on chicory inulin with very high purity, developed to improve texture and mouthfeel in various food applications. Its inulin content ranged from 95 to 99.5%, while in JA it varied between 52% to 75% depending on the harvesting time and the growing environment. Furthermore, commercial inulin has been produced with consideration of microbiological quality control. It is also produced with a long shelf life of at least five years, and the raw materials used were from non-genetically modified food. The chemical composition and the inulin (fructan) content (section 3.2.9) of both sources of inulin were determined (Table 3.1).

Table 3.1: Composition and inulin content of JA flour and CI (mean± SD)

Constituents	**JA flour (g/100g)**	**Commercial inulin Frutafit® TEX (g/100g)**	
	Analysed in present study		Data from the company
Protein	6.91±0.21	Not detected	0
Fats	0.63±0.05	0.32±0.11	0
Moisture	7.56±0.30	6.35±0.28	3
Ash	6.03±0.39	0.36±0.6	0.2
Inulin (fructan)	56.62±1.43	93.29±2.55	96.7

The inulin gel (G) was prepared 24h before manufacturing the doner kebab, using each of AJ powder (JA.P) and CI powder (CI.P) individually by mixing approximately 35g of powder and 100ml of water (~ 25 °C) in a 250ml beaker to obtain suspensions of concentration of 35% (w/w). Samples were then stored at 4 °C in the plastic container and sealed to prevent water loss. Inulin gels from commercial inulin (TEX) can only be obtained in the range of 20-40% (w/w) due to the low water solubility of the inulin powder at room temperature (~ 25 °C) (Chiavaro *et al.*, 2007).

3.2.2 Preparation of doner kebabs

Raw materials used for the production of raw doner kebab were lamb meat (lean meat and fat), sources of inulin (JA flour and CI), water, spice and salt. Lamb meat was purchased from a retailer (Halal To Door, Poole, UK), and the fatty tissue (lamb fat) was manually removed from lean meat and frozen separately at -18 °C until used. Before use they were kept at 4 °C for

approximately 18 h. Lipid and moisture content were determined (section 3.2.4) in lean meat (lipid 7.42%, moisture 71.13%) and lamb fat (lipid 57.1%, moisture 32.8%). Doner kebab meat mixtures were prepared in the food and nutrition lab in samples with added inulin of different types, at different levels, where form and degree of chopping were also factors, according to the formulations in Table 3.2. Pearson's Square was used for the proportion of added lean meat and lamb fat for the targeted fat content in the final raw products. For example, the control product was made without inulin and water, using 657 g (65.6%) of lamb fat and 343g (34.5%) of lean meat to get full-fat content of 40%, which is common in this kind of product (Kilic, 2009).

All other treatments were made with 60% of lean meat and 20% of fat lamb to get 20% fat content in final raw products (50% less fat than control product). The percentage of added non-meat ingredients (JA flour, CI, and water) in relation to meat ingredients (lean meat and lamb fat) in all treatments (except control product) was 20% to 80% respectively in order to replace 50% of fat (Table 3.2).

Both lean meat and fat lamb were minced separately through a 5-mm plate in a mincing machine (Mod EM20, Crypto Peerless mincer, Birmingham, England). Minced lean meat and lamb fat were mixed with salt and spices (~0.5%) in a mixer with spiral dough hook (Professional KitchenAid, Model 5KPM5, Michigan, USA) at medium speed (80 rpm) for 5 min as fine chopping. Two formulations (Table 3.2) were subjected to a very fine chopping and coarse chopping by increasing the time (7min) and speed of chopping (very fine sample) and by decreasing the time (3min) and speed of chopping (coarse sample), otherwise applying the same procedure. After that, the corresponding proportions of non-

meat ingredients were added and then mixed again for 3 min (for coarse treatment), 5min (for fine treatment) and 7min (for very fine chopping treatment). The mixtures of the prepared samples were kept frozen at -18 °C as raw product for further analyses.

Table 3.2: The formulation of doner kebab (g) with different types, levels and form of inulin at different degree of chopping

Degree of chopping	**Treatments**	**Lean meat**	**Lamb fat**	**Water**	**Inulin powder**	**Inulin gel**	**Total batch**
Fine chopping	CP	343	657	----	----	----	1000
	7% JA.P	597	203	130	70	----	1000
	7 % CI.P	597	203	130	70	----	1000
	7% JA.G	597	203	----	----	200	1000
	7% CI.G	597	203	----	----	200	1000
	5% JA.G	149	51	15	----	35	250
	5% CI.G	149	51	15	----	35	250
Very fine chopping	7% JA.P	149	51	32.5	17.5	----	250
Coarse chopping	7% JA.P	149	51	32.5	17.5	-----	250
Fine chopping*	0.5% JA.P	74.3	25.2	----	0.5	----	100
	0.5% CI.P	74.3	25.2	----	0.5	----	100
	1% JA.P	74	25	----	1.0	----	100
	1% CI.P	74	25	----	1.0	----	100
	2% JA.P	73	25	----	2.0	----	100
	2% CI.P	73	25	----	2.0	----	100

CP= control product, JA.P= Jerusalem artichoke powder, CI.P= commercial inulin powder, JA.G= Jerusalem artichoke gel, CI.G= commercial inulin gel, *These treatments were prepared only for inulin determination

3.2.3 Cooking characteristics

The prepared frozen raw samples of doner kebab for all formulations were individually thawed overnight at 4 °C until the meat was soft enough to be cut. Samples were then rolled out and cut into equal shaped slices using a plastic tray and knife with a thickness of 5mm. The slices of doner kebab were weighed and laid on a foil-lined tray, the foil having been weighed beforehand. Samples were cooked using an electric oven (Burco, BC CTCO01 Convection Oven STA, GDPA, Prescot, Merseyside, UK) at 200 °C for 8 min to reach an internal temperature of 72 °C in the centre of the product. The temperature of the oven and the geometric centre of product were monitored continuously using thermocouple probes (K type) (Comark Electronics, Ltd., Littlehampton, UK).

The cooked slices were cooled down at room temperature on a pre-weighed wire rack, over the foil-lined tray they were cooked in, to catch any drip losses of fat and meat juices. Samples were packaged in polyethylene bags and stored in a cool storage at 4±1 °C for further analysis. Samples were weighed before and after cooking. To estimate the cooking yield, and the amount of fat and moisture retained in the samples (Murphy *et al.*, 1975, El-Magoli *et al.*, 1996), the following calculations were performed using equations 3.1, 3.2, and 3.3:

$$\text{Cooking yield (\%)} = \frac{\text{Cooked weight (g)}}{\text{Raw weight (g)}} \times 100 \quad \text{(3.1)}$$

$$\text{Fat retention (\%)} = \frac{\text{Cooked weight (g)} \times \text{Fat (\%) in cooked sample}}{\text{Raw weight (g)} \times \text{Fat (\%) in raw sample}} \times 100 \quad \text{(3.2)}$$

$$\text{Moisture retention (\%)} = \frac{\text{Cooked weight (g)} \times \text{Moisture (\%) in cooked sample}}{\text{Raw weight (g)} \times \text{Moisture (\%) in raw sample}} \times 10 \quad \text{(3.3)}$$

Furthermore, the three raw frozen commercial doner kebab samples purchased from three different kebab shops in Plymouth, UK were used as reference samples (Appendix 3.1). These samples were subjected to cooking characteristics and compositional analysis at the same conditions as for the model system prepared for this study. The purpose of using commercial doner kebabs as references was to compare it with doner kebab samples prepared for this study in term of compositional and cooking characteristics.

3.2.4 Proximate composition and energy values

Cooked samples were used for the determination of moisture, ash, protein, lipid and gross energy. Typically all samples were tested in triplicate according to the standard methods (AOAC, 2003) protocols.

3.2.4.1 Moisture content

All samples were weighed (~ 4g) and dried at 105 °C in a fan assisted oven (Gallenkamp Oven BS, Model; OV-160, Aldridge, England) to a constant weight (approximately 24h), and then cooled to room temperature in a desiccator. Moisture content was calculated using equation 3.4:

$$\textit{Moisture content (\%)} = \frac{\textit{Sample weight (g)} - \textit{Dry weight} \text{ (g)}}{\textit{Sample weight (g)}} \times 100 \quad \text{..... (3.4)}$$

3.2.4.2 Ash

Ash (total mineral or inorganic) content was determined by accurately weighing 500 mg±100mg of well mixed freeze-dried sample into a pre-weighed ceramic crucible. The crucibles and samples were then incinerated in a muffle furnace (Carbolite, Sheffield, England) at 550 °C for 18h until light grey ash results or to constant weight. The inorganic residue in the crucible was the ash of the sample. The percentage of ash was determined from the residue using equation 3.5:

$$Ash\ (\%) = \frac{Sample\ residue\ (g) - Crucible\ weight\ (g)}{Initial\ sample\ weight\ (g)} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.5)$$

3.2.4.3 Lipids

The lipid content was estimated using a rapid soxhlet extraction apparatus (Soxtherm SE- 416, Gerhardt, Bonn, Germany) (Figure 3.2). Briefly, 3g (3 decimal places) of freeze-dried ground sample was accurately weighed into a cellulose thimble and lightly plugged with cotton wool. The thimbles were placed into a wire support and inserted into a pre-weighed beaker. Using a bottle-top dispenser, 140 mL of petroleum ether was added into the beaker that was properly clamped on the heating plate of the Soxtherm unit.

The hot reflux extraction process was carried out following the instruction from the Multistat unit where the extraction setting was moved to the boiling position for 30 min, after which extraction was set to the evaporation position for a further 45 min. At the end of the extraction, the beakers were removed from the unit into a fume cupboard. The thimbles and their holders were removed from

the beaker and under full fume extraction traces of solvent were allowed to evaporate before re-weighing the beakers. Total lipid content was calculated using equation 3.6:

$$\text{Total lipid (\%)} = \frac{\text{Final weight of beaker (g)} - \text{Initial weight of beaker (g)}}{\text{Initial sample weight (g)}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots \quad (3.6)$$

Figure 3.2: Soxhlet system operated in the nutrition laboratory at Plymouth University

3.2.4.4 Protein content

The total crude protein (CP) was determined by the Kjeldahl method measuring the total nitrogen (N) content and using a 6.25 conversion factor. Briefly, 100 mg of well mixed freeze-dried sample was accurately weighed (4 decimal places) directly into a micro Kjeldahl digestion tube along with a catalyst tablet (3g K_2SO_4, 105 mg $CuSO_4.5H_2O$ and 105mg TiO_2; BDH Ltd. Poole, UK) and 10 ml of concentrated sulphuric acid (H_2SO_4) (Sp. Gr. BDH Ltd. Poole, UK).

Digestion was performed with a Gerhardt Kejldatherm digestion block comprising of 40 samples (Gerhardt Laboratory Instruments, Bonn, Germany) with the following schedule; 105 °C for 15 min, 225 °C for 60 min and at 380 °C for 45 min. The digestion process was conducted on a digestion block attached to a scrubber unit (Gerhardt Turbosog unit) in which acid fumes were neutralised with 15% NaOH.

The cooled samples were distilled using a Gerhardt Vapodest 40 automatic distillation unit (Gerhardt Laboratory Instruments, Bonn, Germany) (Figure 3.3), where each sample was diluted with distilled water and neutralised with 37% NaOH. Before boiling the liberated Ammonia in vapour the sample was then condensed into 50 ml of 4% orthoboric acid (H_3BO_3) with 4.5 BDH (as an indicator) by automatically steam distillation. The distillate was then back-titrated against 0.1M H_2SO_4. The protein content was calculated according to the below equation 3.7:

$$\textit{Crude protein (\%)} = \frac{(ST - BT) \times 0.1 \times 14 \times 6.25}{\textit{Initial sample weight (g)}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textit{(3.7)}$$

Where; **ST** is the sample titre (ml); **BT** is the blank titre (ml); 0.1 is the molarities of the acid; 14 is the relative atomic mass of nitrogen, 6.25 is the conversion factor

Figure 3.3: Computerized digestion block (A and B) and distillation unit (C) of the Kjeldahl system at Plymouth University

3.2.4.5 Determination of gross energy

Total caloric values were determined in duplicate using an Adiabatic bomb calorimeter model 1356, (Parr Instrument, Moline, Illinois, USA) (Figure 3.4). The ground dried sample was first compressed into a 1 ± 0.1 g pellet and accurately weighed (4 decimal places). The pellet was then loaded into a nickel crucible with a 10 cm length of fuse wire attached to electrodes, which was formed into a “U” shape to touch the pellet. After having added 1 ml of distilled water to the bomb, sealed the bomb and filled with oxygen to a pressure of 300 psi (20 bars).

A precisely filled bucket of deionised water was used with the instrument to determine the released heat energy after firing. This was weighed at 2000g ± 0.5g prior to loading the bomb.

The crucible was then loaded and sample weigh keyed into the calorimeter for calculation. The result in MJ/kg was printed out, and was later converted to kilocalorie/ kg by this conversion factor: 1MJ/kg = 238.8Kcal/kg.

Figure 3.4: Bomb calorimeter at the nutrition lab at Plymouth University

3.2.5 Physicochemical analysis

3.2.5.1 pH value

The pH values of raw and cooked samples of doner kebabs (10g) were measured directly by using a Microprocessor pH-Meter (pH 213, HANNA Instruments, Ltd, Leighton Buzzard, UK) after previously being calibrated (pH 4.0 and 7.0). The probe was cleaned with distilled water between each reading, to ensure that no fat particles from sample were left in the nozzle which would affect further reading. All determinations were performed in triplicate.

3.2.5.2 Water activity

Water activity (a_w) for both of raw and cooked doner kebabs was measured at room temperature in triplicate using a Novasina Thermoconstanter (TH-2/RTD-33, Zürich, Switzerland), previously calibrated with a solution of saturated NaCl (a_w, 0.75). Sample pots about half full were left in the instrument to obtain constant readings, indicating that equilibrium was reached.

3.2.5.3 Instrumental measuring of colour

A Minolta colorimeter (Minolta Ltd, Model CM2600d, UK) was used to measure the colour [CIE; lightness L*, redness a*, and yellowness b*] of doner samples, where L* is the chrome associated to lightness, a* is the chrome that ranges between green to red and b* is the chrome that ranges between blue and yellow. Samples were measured at five positions on the surface of doner samples and the average reading was recorded. Calibration was made using 10° standard observer and D65 with a white plate.

3.2.5.4 Water holding capacity

Water holding capacity was measured as the water that could not be easily removed from the product matrix by applying a force. A cube of 0.5 g of raw doner samples were placed between two filter papers (Whatman Paper) and two glass plates, and a 10 kg weight was placed on the top glass plate for 5 min. The difference of sample weight before and after procedure represents the water loss and it is expressed as percentage of liberated water in relation to the initial sample weight (Pelicano *et al.*, 2003) according to the equation 3.8:

$$\text{Free water (\%)} = \frac{\text{Weight of free water (g)}}{\text{Weight Initial sample (g)}} \times 100 \quad \text{(3.8)}$$

3.2.6 Instrumental measurement of texture

The texture profile analysis (TPA) using texture analyser (TA-XT2-Stable Micro System, Goldaming, UK) (Figure 3.5) was used to evaluate the samples of cooked doner kebab at room temperature following the method described by Bourne (1978). After cooking, the samples were refrigerated to 4 °C before testing. Sample slices of 0.8 cm thickness, 2.5 cm width and 4 cm length and were compressed twice to 50% of their original height, at a crosshead speed of 2mm/s.

The following parameters were determined: **Hardness** (N or kg) is the maximum force required to compress the sample (H); **Springiness** is the ability of sample to recover to its original form after deforming force was removed (S); **Cohesiveness** is the extent to which sample could be deformed prior to rupture (A2/A1), being A1 the total energy required for the first compression and A2 the total energy required for the second compression; **Adhesiveness** (N s or kg s), work necessary to pull the compressing plunger away from the sample; **Gumminess**, is the force to disintegrate a semisolid meat sample for swallowing (Hardness x Cohesiveness) and **Chewiness,** is work to masticate the sample for swallowing (Springiness x Gumminess) (Bourne, 1978).

Shear strength tests were performed with a Warner-Bratzler reversible blade using TPA. After all samples kept at room temperature, they were cut into slices of 1.6 cm thickness, 2.5 cm width, and 4 cm length for each sample of cooked doner kebab. The crosshead speed was 2mm/s, maximum force to cut the sample (shear force value, kgf) and the work needed to move the blade through the sample (work of shearing, kgf. s) were recorded(Shackelford *et al.*, 1995).

Figure 3.5: Texture analyser unit at Plymouth University

3.2.7 Sensory evaluation

The sensory evaluation protocol was approved by the Human Ethics Committee of the Faculty of Science and Engineering, Plymouth University. Non-trained panellists were recruited among students and staff members at Plymouth University via e-mail invitation (Appendix 3.2) and verbal communication. All of the 28 volunteers were habitual consumers of these types of meat products. Each participant was given the briefing information sheet and a consent form to sign that clearly stated their right to withdraw from the panel at any time and for any reason during the experiment (Appendix 3.3).

Sensory evaluation was based on the British Standards (BS) Guidelines [International Standards Organisation (ISO) 6658-1985] (British Standards 5929, 1986). Standard requirements as explained on the section 4 of Part 1 [general

requirements on BS methods of sensory analysis of foods], were followed as far as possible in regard to materials preparation, test room, and conduct of test in order to ensure that each panellist made an independent judgment.

The panellists were asked to evaluate the following attributes: overall appearance, colour, flavour, texture, chewiness, juiciness, fattiness and overall acceptability, and the description of the attributes were provided (Appendix 3.4). A 9-points balanced hedonic scale (from =1 dislike extremely to 9 like extremely) was used to evaluate the cooked doner kebab samples, with a sensory evaluation form (Appendix 3.5) provided with each sample. Approximately 15 g of each prepared doner sample was reheated in a microwave oven (Brother Hi-Speed Combination Cooker/ Japan) for 30 sec at medium power. Samples were presented at once in no particular order and each coded with three digit random number.

3.2.8 Microstructure analysis of doner kebab (LV-SEM and Cryo-SEM)

Microstructure of doner kebab samples was determined using scanning electron microscope (SEM). Samples were cut from the interior of the meat in pieces of 2–3 mm thickness. Moreover, samples were frozen in liquid nitrogen and left overnight to dry. Then the specimen was mounted on aluminium stubs holders, coated with gold and examined under low vacuum (LV) SEM (LV-SEM, JeoL 5600, Oxford instruments).

SEM (Cryo–SEM -JeoL 6610- Oxford instruments) was also utilised to visualise the microstructure of kebab meat samples with and without inulin. Samples were cut from the central zone of each specimen into 2-3 mm cubes, fixed to a

specimen holder and stuck using Tissue-Tech before being frozen by placing in liquid nitrogen (-190°C). After cryo-fixation, the specimens were directly transferred into Cryo-unit where they were fractured, sublimated for 5 min at -140°C to -90°C and sputter-coated with gold 90 mA for 90s and then transferred into SEM chamber where they were observed. SEM images were taken at different magnifications.

3.2.9 Inulin determination

The Megazyme enzymatic assay commercially available in kit of Fructan HK (Megazyme International, Bray, Ireland) using spectrophotometric technique for determination and quantitation of fructan has been used in this study as described in AOAC method 999.03 (McCleary *et al.*, 2000, Steegmans *et al.*, 2004). The treatments and reference (fructan control flour) samples analysed were the CI (Frutafit® TEX) as supplied (Sensus, Roosendaal, Netherlands) and the prepared JA flour (section 3.2.1).

A range of the prepared doner kebabs were used (Section 3.2.2 and Table 3.2) after being cooked (Section 3.2.3). Some commercial meat products were also subjected to inulin determination such as three commercial pepperoni samples (Peperami Mini Original 10x10g, Peperami Snacks, LSI GmBH, Ansbach, Germany) obtained from local supermarkets (Tesco and Sainsbury, Plymouth, UK) labelled with unknown addition of inulin as the third ingredient in the ingredients list, and the same Peperami samples were spiked in the lab with 3% JA flour and 3% of CI.

All frozen meat products were freeze dried, ground to around 0.5mm particle size and then mixed well to homogenise. All samples were tested in triplicate. The assay procedures were divided into four parts as follows and/or as described in Figure 3.6.

3.2.9.1 Extraction of fructans

A) Samples containing 0-12% fructan (meat products added with inulin): 1g of dry sample and 40ml of hot distilled water (~80ºC) were added into a dry Pyrex beaker (100 ml). **B)** Samples containing 12-100% fructan (JA flour and CI): 1g of dry sample and 400ml of hot distilled water (~80ºC) were added into a dry Pyrex beaker (800 ml). **C)** Fructan control flour with 27.5% inulin (reference): 200mg of dry sample and 80ml of hot distilled water (~80ºC) were added into a dry Pyrex beaker (100 ml).

After that the beakers (**A**, **B**, and **C**) were placed on a hot magnetic stirrer and stirred with heat (around 80°C) for 15 min until the sample was completely dispersed. The solution was cooled down to room temperature and then quantitatively transferred to a volumetric flask and adjust the volume to the mark: **A** (50ml), **B** (500ml), and **C** (100ml) and the contents were mixed thoroughly. An aliquot of the solution was filtered through a Whatman 1 (9 cm) filter circle followed by immediate analysis.

3.2.9.2 Hydrolysis of sucrose and low DP maltosaccharides

Accurately 0.2 ml aliquot of solutions (containing approximately 0.1 to 2.0 mg/ml of fructan) was dispensed into the bottom of glass test-tubes (16 x 100 mm). Then, 0.2 ml of solution 3 (sucrase/maltase mixture) was added to each tube and incubated at 40°C for 30 min, and then 0.5 ml of buffer 2 (100 mM sodium acetate buffer, pH 4.5) was added to each tube with vigorous stirring on a vortex mixer and this is called **Solution A.**

3.2.9.3 Hydrolysis of fructan

Accurately and carefully, 0.2 ml aliquot of **Solution A** (in duplicate) was dispensed to the bottom of plastic spectrophotometer cuvettes (3 ml volume, 1 cm light path), then 0.1 ml of solution 4 (fructanase solution) was added to the bottom of one cuvette **(F+S)**, and 0.1 ml of buffer 2 (100 mM sodium acetate buffer, pH 4.5) was added to the second cuvette **(S).** The contents were mixed thoroughly and the cuvette was covered with Parafilm.

The covered cuvettes were incubated at 40°C for 30 min in a dry hot block heater to effect complete hydrolysis of fructan to fructose and glucose (in the cuvettes containing the fructanase enzyme). 2ml of distilled water (~ 25 $^{\circ}$C), 0.20ml of solution 1 and 0.10 ml of solution 2 were added to both cuvettes **(F+S)** and **(S)** and mixed thoroughly**.**

3.2.9.4 Measurement of fructan

The absorbance was read at 340 nm by spectrophotometer (Camlab, JENWAY, 7315 Spectrophotometer, Bibby Scientific Ltd, UK) at 25°C with **A1** for both cuvettes **(F+S**) and **(S)** after approximate 3 min and the reactions was started by the addition of 0.02ml of suspension 5 (HK/PGI/G-6-PDH) and mixing. After waiting for the end of the reaction (approximately 10-12min) the absorbance of the solution **A2** for both cuvettes **(F+S**) and **(S)** were then read**.** If the reaction had not stopped after 15min, the absorbance continued to be read at 5min interval until the absorbance remained the same over 5min (Figure 3.6 and Appendix3.4).

The inulin content of all samples was then calculated according to the equations that are shown in Appendix 3.4. The amount of inulin obtained from each sample was presented as mean ± standard deviation and expressed as g/ 100g of fresh weight (FW). The amount of fructan present in the sample was expressed in terms of inulin concentration (Simonovska, 2000, Muir *et al.*, 2007, Saengkanuk *et al.*, 2011).

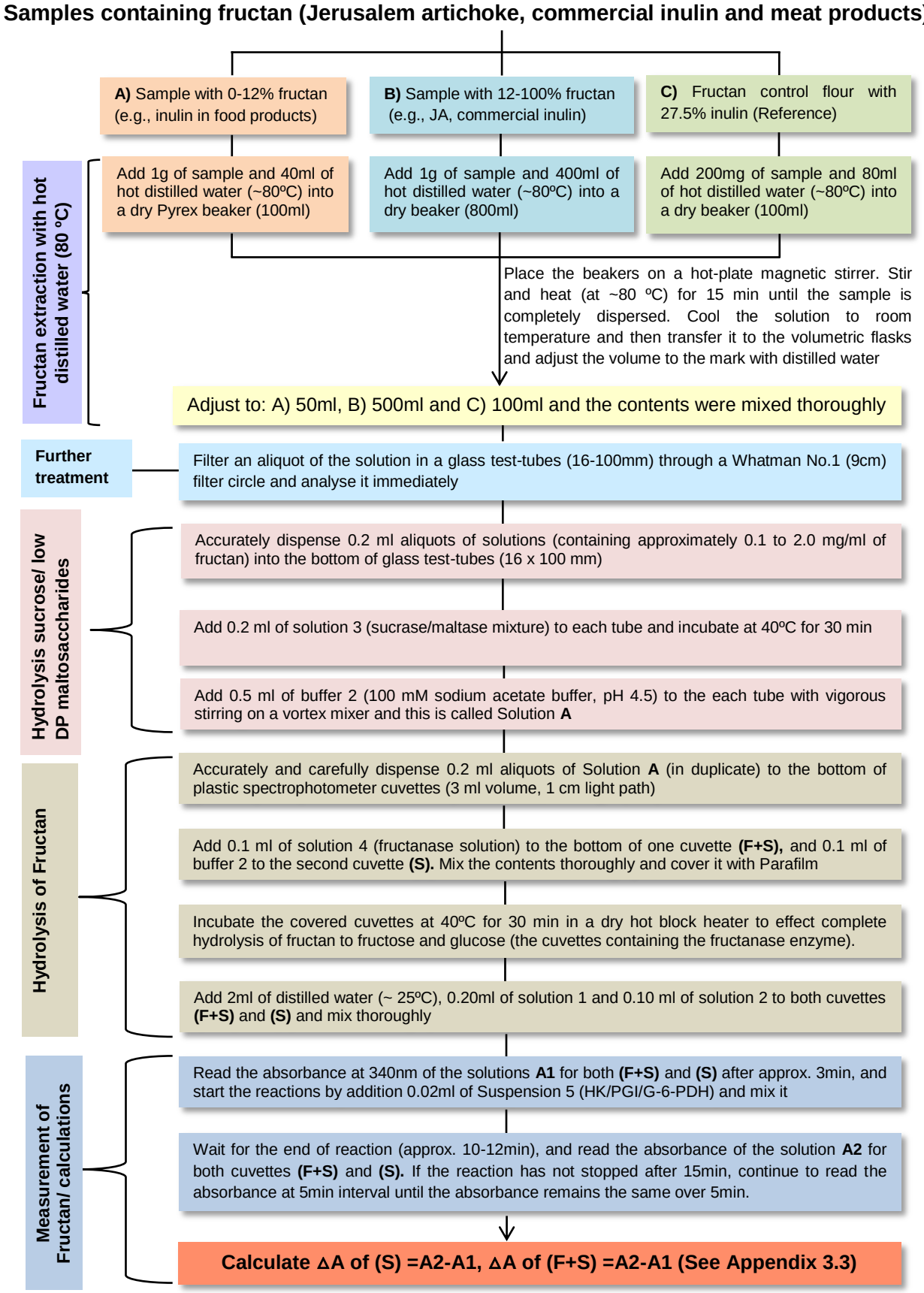


Figure 3.6: The procedure for the Megazyme enzymatic assay (Fructan HK)

3.2.10 Statistical analysis

One-way ANOVA (Analysis of variance) or Kruskal- Wallis tests were used as appropriate. The generated data were analysed by one-way analysis of variance (ANOVA) using Minitab statistics software version 17.0 statistical analysis Minitab v.17 (Minitab Ltd., Coventry). The one-way ANOVA test (Tukey's Multiple Comparison test) was used to identify where significant differences occurred between treatments at the 95% confidence level (associated probability <0.05). Data were presented as mean values ± standard deviation (SD).

However, one-way ANOVA was not appropriate for sensory evaluation in which the data were not normally distributed and not meeting a condition for one way ANOVA. Therefore, Kruskal–Wallis test (non-parametric test) was applied using Minitab statistics software as suggested by (O'Mahony, 1986). The Dunn's test was used to determine significant differences between the different parameters. Data of sensory evaluation are presented as average ranks.

3.3 Results and Discussion

In this study, eight treatments of doner kebabs were prepared with two sources of inulin (JA flour and CI) at different levels, forms, and at different degrees of chopping. The control product was prepared without inulin as reference (Table 3.2). The aim of manufacturing this model system of doner kebab with inulin, but without adding any cereal ingredients was to investigate the effects of inulin exclusively on the physicochemical, cooking characteristics, consumer acceptability and microstructure properties of prepared doner kebab. In commercial doner kebab, manufacturers often use some cereal ingredients such as bread rusk (wheat, gluten), textured soya flour, or wheat flour (Appendix 3.1) that may help to hold some fat juices and water in order to reduce cooking loss, and eventually reduce the cost.

The independent variables (addition of two sources of inulin and degree of chopping) were tested in order to evaluate their impact on the dependent variables such as; the physicochemical, texture, sensory and quality characteristics and microstructure properties of doner kebab. For inulin determination for authentication of labelling purposes, a few samples were selected including (CP, 7% JA.P, 7% CI.P and 5% JA.G). In addition, another six treatments were prepared similarly for inulin determination purposes (Table 3.2).

Using CI Frutafit® TEX in the present study provided an ingredient with long chains or higher molecular weight fractions of inulin (DP ≥22 according to the manufacturers). This kind of inulin can function more like fats when compared to lower degree of polymerisation (DP) fractions. Generally, inulin with higher DP

can be used for fat replacement, while short- chain fructoligosaccharide are of interest as sweeteners (Kays and Nottingham, 2008). For instance, long chain inulin with average DP of about 25 is often used for fat replacement and texture improvement (Saengthongpinit, 2005). Such information should be taken into account for the formulation of inulin enriched products so they fulfil the desired properties, which can be achieved by selecting a type of inulin (Tárrega *et al.*, 2011).

The JA flour was used to investigate their effects as fat replacement on prepared doner samples and to compare to the CI. Generally, most of the published papers reported the use of CI in meat products as source of inulin, while JA was rarely reported. For example, the tubers of JA harvested in KRI have been studied as prebiotic supplement in feed for growth performance of broiler chickens and their effect on overall meat quality attributes (Akoy, 2015). However, its applications in food products has not been investigated yet in KRI, and therefore preparing flours from tubers of JA could be an appropriate technology in area of the world where specialist food ingredients such CI appear to be costly due to currency differences and the costs of importing.

3.3.1 Proximate composition and energy value of cooked doner kebabs

The proximate composition and energy values of the cooked doner samples manufactured with different formulations are presented in Table 3.3. Overall, the moisture content of the prepared doner samples ranged from 48.68–56.26%, which is typical for this type of meat products (Kayisoglu *et al.*, 2003). Similarly, the average moisture content for the three analysed commercial cooked doner

kebab samples in the present study was also in the same range (48.7±2.64) (Table 3.4). Significant (P< 0.05) differences were found between some treatments which could be due to the formulation differences. However, the influence of JA flour and CI was not obvious. Generally, most of the samples with the addition of JA flour and CI had more moisture content compared to the control as they were formulated initially with water, and 50% less fat than control.

Generally, the ash content in the control sample had a similar range to the commercial doner kebab samples analysed in this study as references (Table 3.3 and 3.4). However, all treatments had a lower percentage of ash content compared to the control. This could due to the high content of meat ingredients in the control compared to other batches where 20% of non-meat ingredients added were water and inulin, as inulin contains only traces of ash. The addition of both sources of inulin (JA flour and CI) in doner samples significantly (P< 0.05) decreased protein content (Table 3.3).

For instance, high protein content was observed in the control, amounting to 27.35%. This is due to the high content of meat ingredients in the control product compared to other formulations that contain 20% of non-meat ingredients (inulin and water). Similar results were also reported by Flaczyk *et al.* (2009) and Cegielka and Tambor (2012) who found that addition of inulin significantly (P< 0.05) decreases the content of protein when inulin is used as fat substitute in meatballs and functional chicken burgers respectively.

Furthermore, the amount of protein in commercial doner kebab samples (Table 3.4) analysed in this study was significantly lower than in the control sample

(Table 3.3). One reason, as explained earlier, is that the control sample was prepared with 100% of meat (lean meat and lamb fat), while the commercial doner kebab samples were manufactured with additional ingredients (Appendix 3.1) that have lower protein content.

All analysed doner samples had varied amounts of fat content, resulting from fat content in raw samples as well as from the addition of both sources of inulin as fat substitute. Moreover, due to high variation in cooking loss among formulations, fat contents ranged from 13% to 25%.

Although it was expected that the control would have more fat content, the fat content in the control was only about 19%. This decrease in fat content was due to high loss of fat during cooking process. This observation could be due to the difference in added water which was used to balance the changes. On the other hand, although the control sample had the higher cooking loss, it had the higher added fat and lowest added water resulting to decreased cooking yield.

The average amount of fat content in analysed commercial doner kebab samples was about 26.8g/100g (Tale 3.4). This is a clear indication that manufacturing doner kebabs with some cereal ingredients would retain more fat content than the control sample prepared only with meat.

The variations of fat content can also be explained by variations in the product cooking losses and recorded but uncontrollable experimental errors during sampling and sample preparation, especially during chopping, where some fat could accumulate and be stuck on the top and bottom of the chopper bowl. The addition of inulin in all formulations resulted in products with higher carbohydrate content ranging from 4.1% to 8.78% and it was increased as

concentration of inulin increased, while for the control it was only 0.55% (Table 3.3). This increase in carbohydrates content with addition of inulin was expected and similar trends have been reported (Menegas, 2013). This is because inulin is a carbohydrate that contributes to the increase in total solids (Villegas *et al.*, 2010).

The energy values show proportional relationships with the fat contents for most of the treatments. Although higher fat content and higher energy were expected for the control and vice versa for the rest of the treatments, there was some loss of fat during cooking and in addition to experimental errors. Inulin contributes only about 1.5 $kcal.g^{-1}$ which is used on food labelling (Kays and Nottingham, 2008).

Table 3.3: Proximate composition (%) of experimental treatment batches of cooked doner samples (mean± SD)

Batch	Moisture %	Ash %	Protein %	Fat %	Carbohydrate%	Energy Kcal/100g
CP	50.3±1.1^{bc}	2.7±0.60^{a}	27.3±0.81^{a}	19.0±0.72^{d}	0.5	319.36
7% JA.P	52.0±1.7^{abc}	2.4±0.55^{ab}	18.8±0.30^{d}	18.8±0.14^{d}	7.7	302.22
7% CI.P	49.9±2.32^{bc}	1.6±0.75^{abc}	21.0±0.82^{b}	21.6±0.20^{b}	5.7	346.21
7% JA.G	52.6±1.01^{abc}	2.3±0.63^{ab}	18.9±0.10^{de}	18.9±0.10^{d}	7.1	301.94
7% CI.G	48.6±1.48^{c}	0.8±0.47^{c}	19.4±0.62^{bc}	25.3±0.82^{a}	5.6	332.82
5% JA.G	54.3±0.65^{abc}	1.6±0.59^{abc}	19.1±0.66^{d}	20.8±1.14^{bc}	4.1	298.77
5% CI.G	56.2±3.84^{a}	1.1±0.29^{bc}	17.3±0.11^{e}	20.1±0.33^{bcd}	4.9	291.75
7% JA.P*	55.5±0.86^{ab}	1.8±0.26^{abc}	20.8±0.09^{bc}	13.0±0.25^{e}	8.7	254.21
7% JA.P×	51.6±2.79^{abc}	2.0±0.28^{abc}	18.5±0.50^{de}	19.2±0.11^{bc}	8.5	304.29

$^{a-e}$Mean in the same column with different superscripts are significantly different (P <0.05). *very fine chopping, ×coarse chopping

Table 3.4: Proximate composition (%) of cooked commercial doner kebab samples (mean ± SD)

Kebab shops	Moisture %	Ash %	Protein %	Fat %
Shop1	49.2±0.81	3.1±0.31	17.8±0.34	23.4±0.9
Shop2	51.1±0.21	2.6±0.35	15.7±1.13	22.9±0.2
Shop3	45.8±0.68	2.4±0.06	15.5±0.89	34.0±1.0
Average	48.7±2.64	2.68±0.34	16.32±1.28	26.78±6.26

3.3.2. Physical properties of raw and cooked doner samples

Generally, the introduction of inulin sources in doner samples led to significant increase ($P<0.05$) in the pH values in all cooked samples ranging from 6 in the control up to 6.45 in uncooked samples supplemented with JA. These increases were more significant in samples with JA compared to commercial inulin in all treatments (Table 3.5). Moreover, there was a consistent increase of pH value from the uncooked to the cooked samples in all treatments.

These increases of pH values, especially with JA compared to commercial inulin, could be explained in that the pH of commercial inulin produced from chicory is about 5 to 7 (Kays and Nottingham, 2008). In contrast, a study by Abou-Arab *et al.* (2011) found that the pH values of inulin powder produced from JA tubers is about 6.7 to 6.9.

Another reason that could explain the increase of pH levels in treatments compared to the control is the addition of water in all treatments compared to the control which only consisted of lean meat and lamb fat (Table 3.2). However,

many studies indicated that the reduction of fat and the addition of inulin from chicory did not affect the pH of the fermented sausages (Mendoza *et al.*, 2001, Salazar *et al.*, 2009, Menegas, 2013, Keenan *et al.*, 2014).

Generally, the water activity of raw meat products is greater than in cooked products. For instance, observed data in Table 3.5 ranged from 0.94 to 0.96 in raw and 0.92 to 0.96 in cooked samples. However, addition of inulin did not affect the water activity for both raw and cooked samples compared to control. Similar results were reported by several authors (Mendoza *et al.*, 2001, Cáceres *et al.*, 2004, Nowak *et al.*, 2007, Menegas, 2013) that inulin had no effect on water activity in German bologna- type sausages, dry fermented sausages, dry-fermented chicken sausages and cooked meat sausages respectively.

The water holding capacity was measured to understand the degree of binding that the meat and gel structure had, with the CI powder sample holding about 30 g per 100 g of product in a similar range to that of the control, while the others hold significantly more water- up to 50% more for JA samples, regardless of the degree of chopping (Figure 3.7). Colour measurement is one of the important characteristics in raw and cooked meat products. The instrumental colour measurements (lightness (L^*-value), redness (a^*-value), and yellowness (b* -value) of uncooked and cooked doner samples of the different formulations were affected by addition of inulin form (powder and gel) and types (Table 3.6). The differences in colour parameters are due to the characteristics of the inulin colour added to the meat products.

In the uncooked doner samples, the lightness (L^*) was increased with samples formulated with CI in form of gel, and it was significant ($P< 0.05$) for treatment

with 7% CI.G compared to control and other treatments. In the cooked doner samples, there was also a significant ($P< 0.05$) increase in the lightness (L^*) value of all treatments enriched with CI compared to control and some treatments with JA. For instance, the highest value of lightness was observed with 7% CI.G and then by 5% CI.G (Table 3.6). A similar trend of increasing the values of L^* by addition of 3% of inulin in cooked chicken products was observed (Cava *et al.*, 2012).

This increase in lightness could be due to the white colour of commercial inulin reflected on the both uncooked and cooked samples, whereas the colour of JA powder is white or light-gray (Saengthongpinit, 2005), or brown to yellowish (Bekers *et al.*, 2007), depending on purity, preparation methods, and concentration of JA powder when used as gel (Saengthongpinit, 2005). As a result, a lower value of lightness was found in all samples incorporated with JA in form of powder and gel compared to CI.

The introduction of inulin significantly ($P< 0.05$) lowered the redness (a^*) compared to the control sample in uncooked and to some extent in cooked samples. Similar results were also reported by Cava *et al.* (2012). The Yellowness b* seems to be changeable in raw, cooked samples and between control and treatments. However, there was no clear trend regarding the addition types and form of inulin, but in general they were lower than control.

Table 3.5: pH and water activity values of uncooked and cooked doner samples

Batch	pH		a_w	
	Uncooked	Cooked	Uncooked	Cooked
CP	5.98±0.01^{ef}	6.0±0.19^{b}	0.96±0.01^{a}	0.92±0.00^{a}
7% JA.P	6.15±0.01^{c}	6.43±0.03^{a}	0.96±0.01^{a}	0.95±0.02^{a}
7% CI.P	5.95±0.01^{f}	6.26±0.05^{a}	0.95±0.01^{a}	0.95±0.01^{a}
7% JA.G	6.23±0.01^{a}	6.45±0.04^{a}	0.94±0.00^{a}	0.94±0.03^{a}
7% CI.G	6.0±0.00^{e}	6.34±0.05^{a}	0.96±0.01^{a}	0.94±0.02^{a}
5% JA.G	6.08±0.01^{d}	6.35±0.02^{a}	0.95±0.01^{a}	0.95±0.02^{a}
5% CI.G	5.97±0.01^{ef}	6.25±0.02^{a}	0.96±0.01^{a}	0.95±0.01^{a}
7% JA.P*	6.2±0.01^{b}	6.43±0.02^{a}	0.96±0.01^{a}	0.96±0.02^{a}
7% JA.P×	6.18±0.01^{b}	6.39±0.01^{a}	0.95±0.01^{a}	0.95±0.03^{a}

All values are mean ± standard deviation of three replicates. $^{a-f}$Mean in the same column with different superscripts are significantly different (P <0.05). *very fine chopping, ×coarse chopping

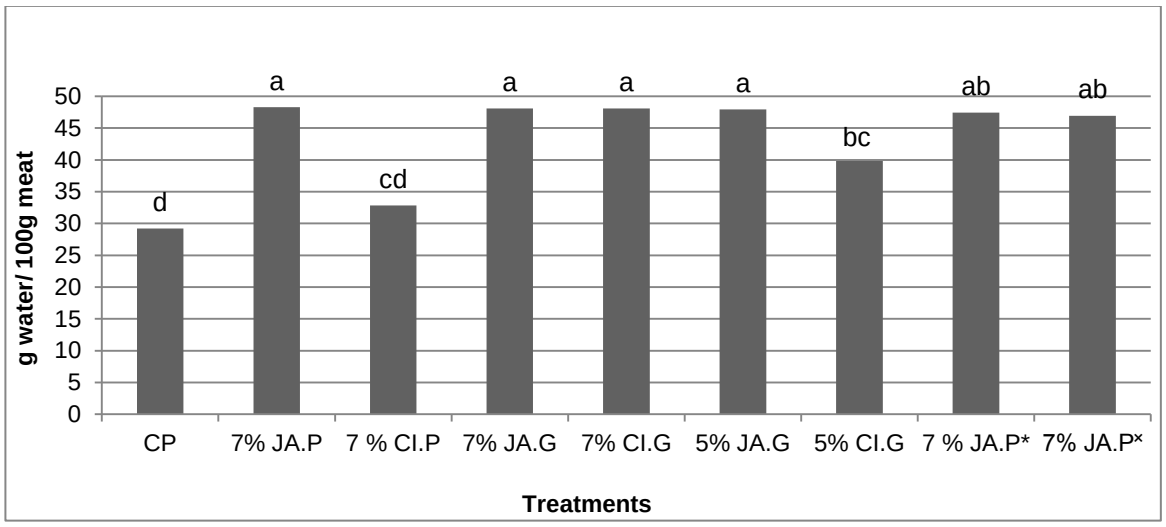


Figure 3.7: Comparison of water holding capacity of all treatments (raw samples) expressed as g water/100g meat

Table 3.6: Colour measurements for uncooked and cooked doner samples

Batch	Uncooked			Cooked		
	Lightness (L^*)	Redness (a^*)	Yellowness (b*)	Lightness (L^*)	Redness (a^*)	Yellowness (b*)
CP	63.07±1.03^{b}	8.67±1.07^{a}	17.06±1.3^{ab}	50.25±2.25^{d}	3.56±0.67^{ab}	5.11±1.46^{bc}
7% JA.P	57.83±0.91^{cd}	6.32±0.21^{bc}	17.41±0.28^{a}	56.72±2.14^{b}	3.31±0.10^{ab}	8.73±1.51^{a}
7% CI.P	59.47±0.91^{cd}	6.21±0.26^{b}	16.36±0.39^{ab}	55.72±3.73^{bc}	3.11±0.23^{b}	6.55±1.76^{ab}
7% JA.G	57.63±1.15^{d}	5.41±0.25^{cd}	16.42±0.42^{ab}	51.05±0.99^{d}	3.53±0.49^{ab}	2.00±1.09^{d}
7% CI.G	67.17±0.45^{a}	5.32±0.34^{d}	16.16±0.85^{ab}	58.74±1.13^{ab}	4.12±0.29^{a}	8.24±0.48^{a}
5% JA.G	54.36±1.33^{e}	5.12±0.44^{d}	13.9±1.15^{c}	52.13±1.29^{cd}	3.59±0.33^{ab}	4.44±0.5^{bc}
5% CI.G	63.83±0.77^{b}	5.68±0.55^{cd}	17.1±0.68^{ab}	61.79±2.61^{a}	3.45±0.42^{ab}	8.17±1.18^{a}
7% JA.P*	55.15±0.60^{e}	5.48±0.22^{cd}	15.84±0.35^{b}	50.38±1.11^{d}	3.78±0.49^{ab}	3.32±0.88^{cd}
7% JA.P×	59.66±0.76^{c}	5.36±0.19^{cd}	16.4±0.60^{ab}	50.49±1.00^{d}	3.91±0.24^{ab}	4.01±0.66^{cd}

All values are mean ± standard deviation of five replicates. Values in columns with different letter are significantly different ($P < 0.05$). *very fine chopping, ×coarse-chopping

3.3.3 Cooking characteristics of doner kebab

The measurements of cooking yield, fat retention and water retention were significantly improved by addition of both sources of inulin compared to control as given in Table 3.7. Moreover, the highest cooking loss, lower fat and moisture retention were observed on the control sample formulated without inulin. Hughes *et al.* (1997) reported that cooking yield was affected by the amount of fat and water content. Reducing fat content on formulated doner samples and replacing with inulin dietary fibre resulted in reduced cooking loss compared to the control product. Significant improvements on cooking yield, fat retention and water retention were obtained (Mahmoud and Badr, 2011) when wheat bran fibres were added as partial replacement of beef on beef burger. Gök *et al.* (2014) also found significant decrease ($p< 0.05$) in cooking loss when textured soy protein in combination with tomato pulp was added into ground chicken doner kebab.

This decrease in cooking yield, fat and water retention can be explained in that the excessive fat on the control sample was separated and the water released during cooking as the formulation was not added with any fibres or cereals ingredients. Generally, dietary fibres tend to keep moisture and fat in the meat matrix. This phenomenon is well documented by several authors who used different types of dietary fibres on meat products (Besbes *et al.*, 2008, Choi *et al.*, 2010, Mahmoud and Badr, 2011).

Furthermore, the cooking characteristics of commercial doner kebabs were also analysed in the present study (Table 3.8). Significantly higher cooking characteristics were observed in commercial doner kebabs compared to the prepared model system of doner kebab, without and with inulin. This higher

observation in commercial doner samples could be due to the preparation method followed by manufacturers to reduce the cost and the ingredients used (Appendix 3.1) such as cereals, emulsifier and stabilisers that may help to hold the structure and eventually reduce cooking yield, and retain water and fat.

In the present study, the highest cooking yields were obtained from samples formulated with 7% JA.G. Generally, the inclusion of inulin in the form of gel increases cooking yield, fat retention, and water retention more than when is added as powder. In contrast, Álvarez and Barbut (2013) found that that inclusion of inulin powder had a higher contribution towards emulsion stabilization and had significantly ($P < 0.05$) reduced cooking loss compared to inulin gel.

Furthermore, the effect of chopping degree also affected the cooking properties (Figure 3.8). For instance, higher values of cooking yield, fat and water retention were obtained from formulation subjected to fine chopping followed by coarse chopping compared to very fine chopping with the same type, level and form of inulin inclusion.

Table 3.7: Effect of inulin powder and gel on the cooking properties of doner samples

Cooking characteristics	**CP**	**(%) Inulin in form of powder**				**Inulin in form of gel (%)**			
		7% JA	7% CI	7% JA*	7%JA×	7% JA	7% CI	5% JA	5% CI
Cooking yield	46.8	74.27	64.05	66.75	73.5	82.74	70.76	74.43	77.98
Fat retention	22.22	69.81	69.17	43.39	70.57	78.2	89.52	77.4	78.37
Moisture retention	51.16	61.65	51.01	59.10	60.54	69.43	54.96	62.62	67.89

*coarse chopping, *very fine chopping, CP= control product

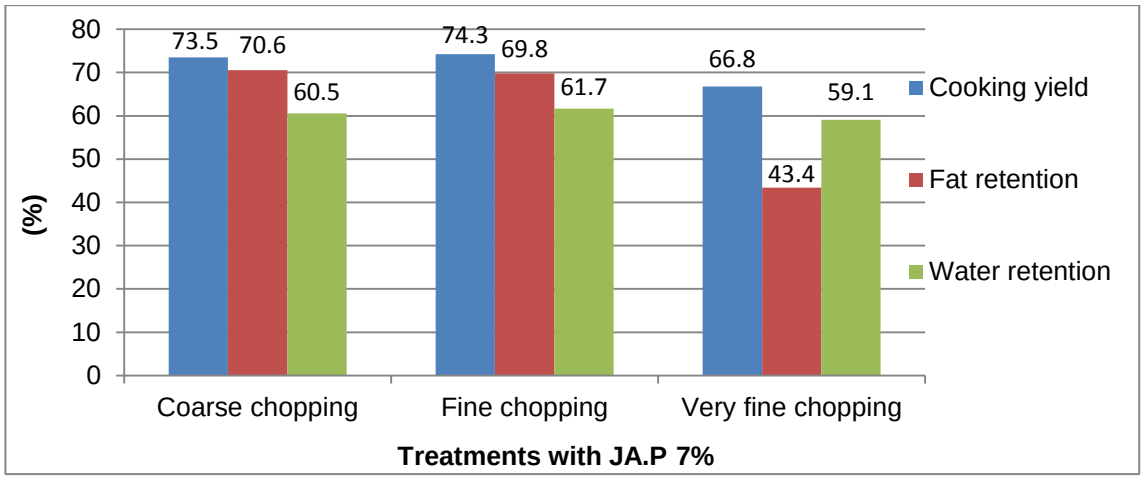


Figure 3.8: Effect of chopping degree on the cooking properties of doner samples

Table 3.8: Cooking characteristics of commercial doner samples

Cooking characteristics	Kebab shop1	Kebab shop2	Kebab shop3	Average
Cooking yield	85.64	84.12	90.14	86.65±3.11
Fat retention	72.92	77.37	86.71	79.00±7.04
Water retention	82.10	79.45	85.82	82.45±3.20

3.3.4 Texture analysis

The results of the TPA in experimental cooked doner samples are shown in (Table 3.9). Hardness, as one of the most important attributes for texture and sensory evaluation (Mittal and Barbut, 1994), was changed due to the addition of inulin. Inulin in the form of gel caused softening to the doner samples formulated with inulin-gel in both sources of inulin compared to the same type of inulin when it is added in form of powder. The formulation with 7% JA.P resulted

in more hardness and was significantly (P< 0.05) higher than 7% JA.G. This amount of inulin (7%) in the form of powder is sufficiently high to make the product harder. Therefore, in order to avoid possible sensory alteration from excessive hardness, inulin powder could be limited up to 7% to be added in meat products. Similar results were obtained by Selgas *et al.* (2005) who used inulin gel and powder as functional ingredients in cooked meat sausages. Moreover, Luisa García *et al.* (2006) also claimed that the inclusion of inulin, when added as powder, tends to increase the hardness, while it cause softness when inulin is added as gel.

Gumminess and chewiness are dependent on hardness therefore similar observation trends were obtained and they are considered as secondary parameters of texture (Luisa García *et al.*, 2006). Less chewiness of products were observed with treatments when inulin was added in the form of gel and lower levels compared to the control and higher level of inulin. Therefore, the decrease in chewiness and hardness in batches formulated with inulin-gel tend to produce softer doner samples that are easier to chew. These results were in line with the findings by Luisa García *et al.* (2006) regarding the effect of inulin in both forms (powder and gel) on the textural properties of mortadella.

Springiness, cohesiveness, and adhesiveness were slightly modified without significant differences (P> 0.05). Chopping degree had no significant (P> 0.05) differences on the textural properties and shear force (Table 3.9) in the three formulations that were chopped with different degrees with the same amount (7%) of added JA powder (Table 3.2).

On the other hand, the reduction in fat content and the addition of both sources of inulin caused a decrease in shear force. This means more tenderness with samples that were formulated with inulin compared to control (Table 3.9). For example, research classified the tenderness of cooked bovine muscle into the following categories according to their shear force values; very tender (shear force less than 3.2 kgf), tender (3.3 to 3.9 kgf), intermediate (3.9 to 4.6 kgf), and tough when it's more than 4.6kgf (Belew *et al.*, 2003, Ishihara *et al.*, 2013). Therefore, most of the results obtained in this study were within very tender to tender ranges, except the value of control that was 4.1kgf which can be classified as intermediate tender.

These texture parameters were defined physically and known as instrument texture in sensory of consumers' point of view. Testing texture instruments can detect and quantify only certain physical parameters, which then must be interpreted in terms of sensory perception (Szczesniak, 2002). Therefore, sensory evaluation was carried out to measure some important quality parameters as discussed in the below section.

Table 3.9: Influence of inulin inclusion on the textural parameters and shear force of doner kebab

Batch	Hardness (kg)	Springiness	Cohesiveness	Adhesiveness Kg s	Gumminess	Chewiness	Shear force (Kgf)
CP	2.18±0.13^{ab}	0.77±0.07^{a}	0.54±0.05^{a}	-0.09±0.12^{a}	1.17±0.11^{ab}	0.92±0.16^{ab}	4.10±1.49^{a}
7% JA.P	2.94±0.58^{a}	0.77±0.04^{a}	0.51±0.01^{a}	-0.20±0.35^{a}	1.52±0.32^{a}	1.18±0.21^{a}	3.34±0.96^{ab}
7% CI.P	1.94±0.52^{ab}	0.80±0.05^{a}	0.51±0.03^{a}	-0.30±0.40^{a}	0.99±0.28^{abc}	0.80±0.23^{ab}	3.35±1.73^{ab}
7% JA.G	1.79±0.32^{b}	0.81±0.06^{a}	0.51±0.02^{a}	-0.02±0.04^{a}	0.92±0.12^{bc}	0.74±0.04^{ab}	2.30±0.32^{ab}
7% CI.G	2.19±0.53^{ab}	0.74±0.03^{a}	0.49±0.03^{a}	-0.15±0.08^{a}	1.09±0.34^{abc}	0.82±0.30^{ab}	3.08±0.24^{ab}
5% JA.G	1.97±0.10^{ab}	0.83±0.05^{a}	0.52±0.02^{a}	-0.44±0.39^{a}	1.04±0.07^{abc}	0.87±0.11^{ab}	2.16±0.38^{ab}
5% CI.G	1.15±0.28^{b}	0.77±0.01^{a}	0.51±0.02^{a}	-1.07±1.85^{a}	0.58±0.12^{c}	0.45±0.08^{b}	1.84±0.61^{b}
7% JA.P*	2.01±0.24^{ab}	0.83±0.02^{a}	0.53±0.01^{a}	-0.31±0.30^{a}	1.07±0.11^{abc}	0.89±0.07^{ab}	2.91±0.55^{ab}
7% JA.P×	2.14±0.24^{ab}	0.75±0.01^{a}	0.49±0.02^{a}	-0.40±0.57 ^{a}	1.05±0.06^{abc}	0.80±0.04^{ab}	2.16±0.34^{b}

Values are given as mean ± standard deviation, ANOVA followed by Tukey's test with different letters $^{a-c}$ within the column are significantly different ($P < 0.05$).

*very fine chopping, ×coarse chopping

3.3.5 Sensory evaluation

Sensory evaluation is one of the most common and useful measurement to assess the quality of processed meat products (Heinz and Hautzinger, 2009). It is an important step for new product development that measures consumer preference (Guàrdia *et al.*, 2006). It is crucial to assess the sensory characteristics of a food product for consumers. However, often food industries face challenges in providing desired sensory properties, whether by modifying an existing product, designing new product development and even when raw materials or processing devices are modified (Curt *et al.*, 2004).

The most common rating scale used in the food industry for assessing like and dislike of a food product is the 9-point hedonic scale where the panellists are asked to rate samples from dislike extremely (1) to like extremely (9) (Peryam and Pilgrim, 1957). Before evaluating sensory attributes in the present study, the panellists were given no information on the level of fat content in the products because such knowledge can influence rating scores (Kähkönen and Tuorila, 1998, Hamilton *et al.*, 2000).

In the present study, 28 panellists were participated to assess their product preferences, and the 9 point hedonic scales was used which requires 20 or more assessors according to the BS (British Standards 5929, 1986), when considering intensity of the products. Therefore, 28 panellists were enough and valid according to BS, but when considering the degree of preferences more panellists were required.

The ratings of each sensory attribute were converted to numerical scores and the numerical scores were collected for statistical analyses. Due to the unequal

interval nature of the 9-point hedonic scale, that reduces the mathematical level of the data obtained using the scale to ordinal, non-parametric statistics such as Kruskal- Wallis test was used to analyse the data (Jaeger and Cardello, 2009). The average rank of 28 panels evaluation from each sensory attributes are shown in Figure 3.9 and pairwise comparisons for the sensory attributes were used for comparison between treatments (Figure 3.10). Spider plot (Figure 3.11) was used to visualize the mean rating scores for various sensory attributes and to show the differences between control and treatments containing sources of inulin.

From the eight sensory attributes evaluated, flavour, texture, and chewiness had no significant differences obtained ($P > 0.05$), which indicate neither addition of inulin sources nor fat reduction had any significant effect on these attributes. However, treatments with JA had higher scores compared to other treatments. Often similar reduced-fat meat samples such as sausage experienced reduction in flavour (Homer *et al.*, 2000). This is due to the lack of some fat-soluble compounds, which, when released during chewing, contribute to the overall flavour (Devereux *et al.*, 2003).

Another five attributes (overall appearance, colour, juiciness, fattiness and overall acceptability) scored significant differences ($P < 0.05$) among some analysed samples. The two treatments that were formulated with JA flour in form of powder and gel (7% JA.P and 7% JA.G) obtained the highest quality scores of average rank compared to control and CI (Figure 3.9 and 3.10). This means that 50% of fat replacement in doner samples not only does not negatively influence overall acceptability, but also improved most sensory quality attributes. Therefore, this was a clear indication that panels considered

the addition of JA in both forms more acceptable in sensory point of view compared to the control and CI inclusion. Their positive impacts were noticed in all sensory attributes. Similar results were obtained by Gedrovica and Karklina (2013) who found that addition of JA powder had significant ($p< 0.05$) impact on the appearance, taste, texture, colour and juiciness of meatballs.

CI in both forms had a lower average rank compared to control in all attributes without significant differences ($p> 0.05$). However, the scores obtained from CI were all in the acceptable region of liking. In contrast, the improved sensory quality attributes through commercial inulin inclusion were reported by Cegielka and Tambor (2012) who studied the effect of adding inulin as functional food ingredient in chicken burgers. Moreover, Flaczyk *et al.* (2009) also found an improvement on the texture of meatballs when inulin was added as fat replacer.

Addition of JA powder appeared to have a positive effect on the overall appearance, being rated higher than control and CI, while CI had a lower average rank compared to control, but none of the treatments were significantly different ($p> 0.05$). CI in form of gel had a significantly ($P< 0.05$) lower average rank in the attribute of colour compared to other treatments. This could be due to the white colour of CI, compared to the yellowish to brown colour of JA which is closer to the colour of meat (Bekers *et al.*, 2007) (Figure 3.1). Additionally, JA makes the colour of meatballs darker (Gedrovica and Karklina, 2013).

The juiciness of reduced-fat doner samples is more likely to be rated as higher than average rank compared to control with only significant differences ($P< 0.05$) in JA.G compared to control. This was a clear indication that JA in both forms holds moisture. Juicier meatballs that hold more moisture were also

manufactured with the addition of JA powder compared to control (Gedrovica and Karklina, 2013).

Often acceptability of foods is related to their fat content, especially in meat products. Moreover, high palatability is also associated with high fat content (Kähkönen and Tuorila, 1998, Hamilton *et al.*, 2000). However, in the present study, when both sources of inulin were added to replace 50% of fat, no significant reduction on overall acceptability was found. There was even an improvement, especially with 7% of JA gel and powder which scored the highest for overall acceptability.

Finally, it can be concluded that all sensory attributes contributed to the overall acceptability of the prepared samples with JA, whereas CI in both forms had only higher scores on chewiness and juiciness, while the overall acceptability and other attributes had a lower rank.

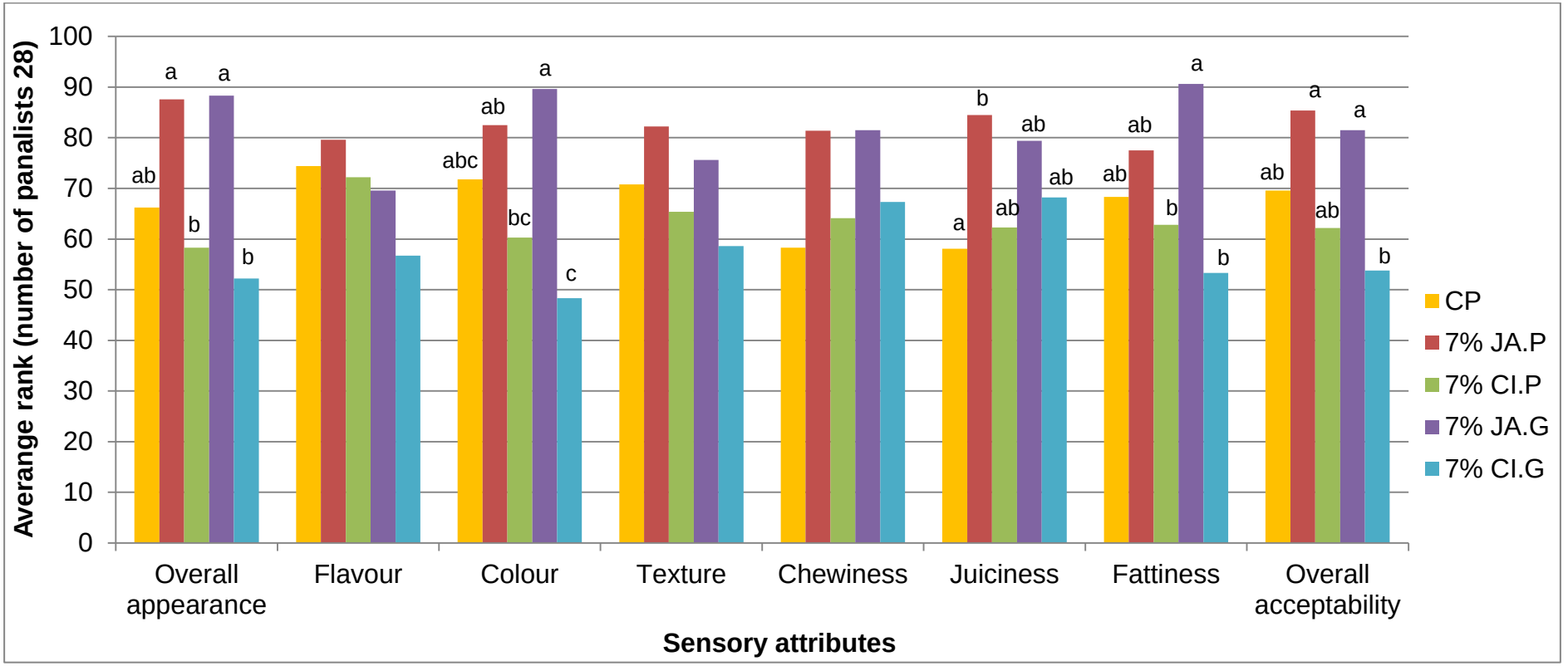


Figure 3.9: Average rank of the eight sensory attributes evaluated (n=28) of prepared doner kebabs with and without added powder and gel of the JA flour and CI. Pairwise comparisons followed by using Dunn's test with different letters a-c within one attribute are significantly different ($P < 0.05$)

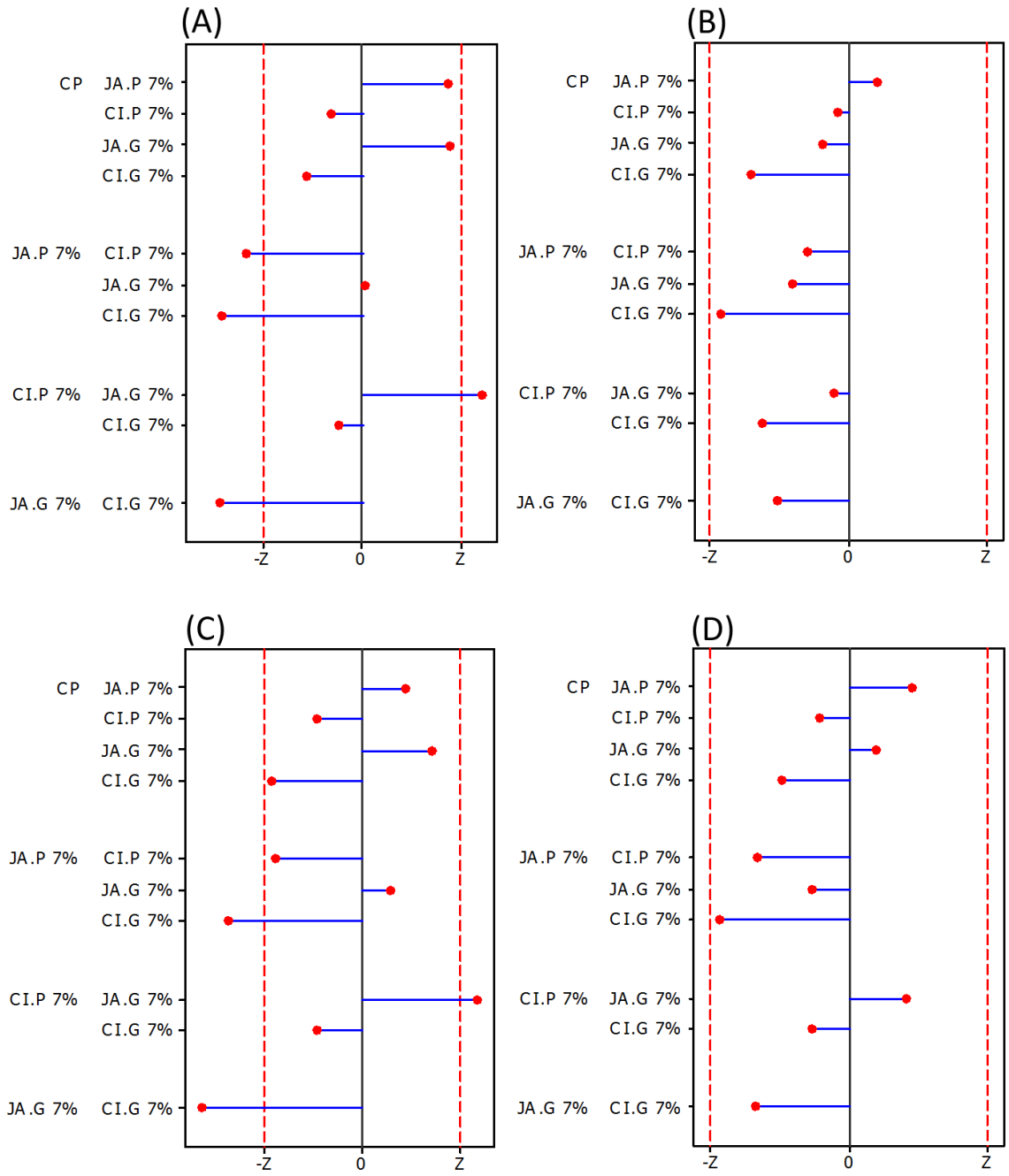


Figure 3.10A: Pairwise comparisons (normal (0,1) distribution) of the sensory attributes of prepared doner kebabs using Dunn's test (Bonferroni Z-value: 2.326). (A) Overall appearance, (B) Flavour, (C) Colour, (D) Texture

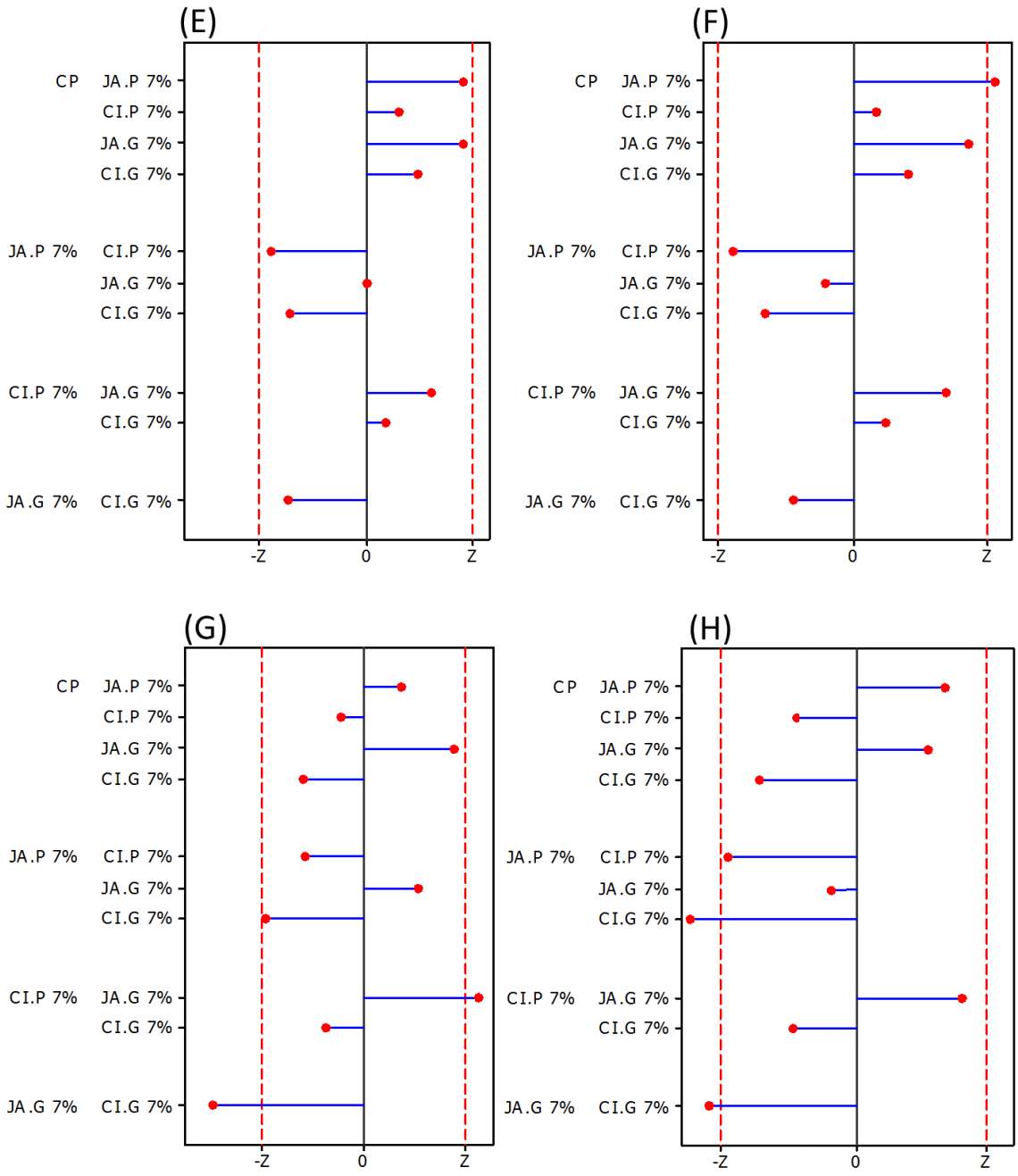


Figure 3.10B: Pairwise comparisons (normal (0,1) distribution) of the sensory attributes of prepared doner kebabs using Dunn's test (Bonferroni Z-value: 2.326). (E) Chewiness, (F) Juiciness, (G) Fattiness, (H) Overall acceptability

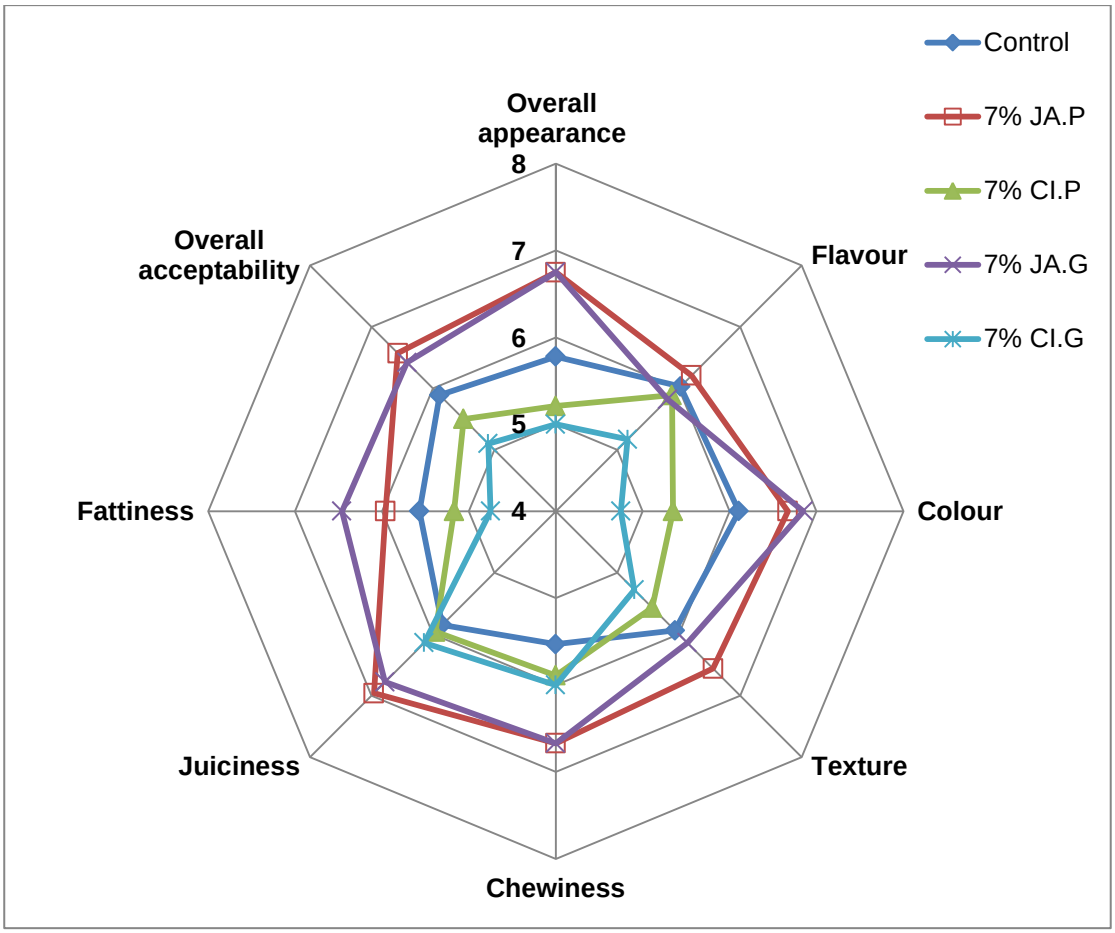


Figure 3.11: Sensory evaluation of doner kebab samples with overall appearance, flavour, colour, texture, chewiness, juiciness, fattiness and overall acceptability based on hedonic 9 scales (n=28)

3.3.6 Qualitative changes in microstructure of doner kebab samples

The relationship between the quality of meat and meat products and their microstructure is a known fact (Larrea *et al.*, 2007). The ability of meat to retain its original and added moisture is economically very important for the food industry. This is due to the fact that water is a major constitute of meat, consisting of about 70g/100g of lean meat (Barbut, 2006).

Furthermore, any amount of moisture lost during cooking will negatively affect product yield and other quality attributes such as favour, tenderness and texture (Offer *et al.*, 1984, Tsai *et al.*, 1998). Therefore, examining the micrographs of comminuted meats systems and mechanisms responsible for the formulation of final structural could help to better understand quality (Atughonu *et al.*, 1998).

Meat products with dietary fibres added as a fat replacer or functional ingredients have been subjected to the examination of microstructure (Comer *et al.*, 1986, DeFreitas *et al.*, 1997, Morin *et al.*, 2004, Barbut, 2006, Álvarez and Barbut, 2013). However, reports on the potential impacts of addition of inulin on the microstructure of doner kebab products using SEM techniques are rarely available. Therefore, Cryo-SEM and LV-SEM images were used as helpful tools to better understand the influence of reducing fat by addition of sources of inulin in forms of powder and gel.

Testing the changes in microstructure, in particular inulin gelatinisation and the matrix between starch-protein in relation to the product structure quality, could link to other quality characteristics such cooking yield, texture and sensory attributes. The micrographs (Figure 3.12) from Cryo-SEM were examined for

the microstructure effects of cooked samples resulting from the addition of inulin (powder and gel) of JA and CI at level of 7% compared to control (without inulin).

Samples with 7% of JA.G (B) and 7% CI.G (D) had a clear gelling network and the meat matrix showed some elongated fat globules within matrix components with irregular shapes that could be due to addition of inulin and preparation methods including mixing and chopping. This gel network holds moisture and can be linked to the high reduction of cooking loss and the production of a softer texture compared to same level of powder and to the control, especially with JA as explained earlier (Section 3.3.3and 3.3.4).

In contrast, the samples with 7% JA.P (A) and 7% CI (C) had a partial gelatinised network with more available matrix spaces for filling and show a high swelling capacity. As a result, JA.P in particular provided a texture harder than JA.G (Table 3.7). The effect of the preparation methods of SEM techniques on the microstructure of cooked samples can be seen from the micrographs (Figure 3.13) using LV-SEM and Cryo-SEM.

In general, more shrinkage and fragile appearance can be seen from LV-SEM, and this could be due to the water being removed from the sample preparation step after freeze-drying overnight which causes shrinkage in the microstructure. The physical appearance of more spongy structure on Cryo-SEM when compared to LV-SEM technique was observed. Fat substituted with non-meat ingredients influenced the structure, and indicates the strength of the protein-gel matrix to determine their effects on the cooking characteristics and eating quality of the final products (Morin *et al.*, 2004).

Unlike the microstructure of the control, the micrographs with added inulin have a spongier gel type structure compared to the control sample, with tightly structured muscle fibres. Although chopping degree had no significant effects on cooking characteristics and eating quality, fine chopping had a uniform structure with numerous small pores, or open spaces, which would probably result in more absorptive capacity and better water holding capacity compared to coarse structures with large spores and very fine chopping (Figure 3.14) (Hermansson, 1998). Moreover, the physical state of the fat globules during chopping could affect their size and distribution.

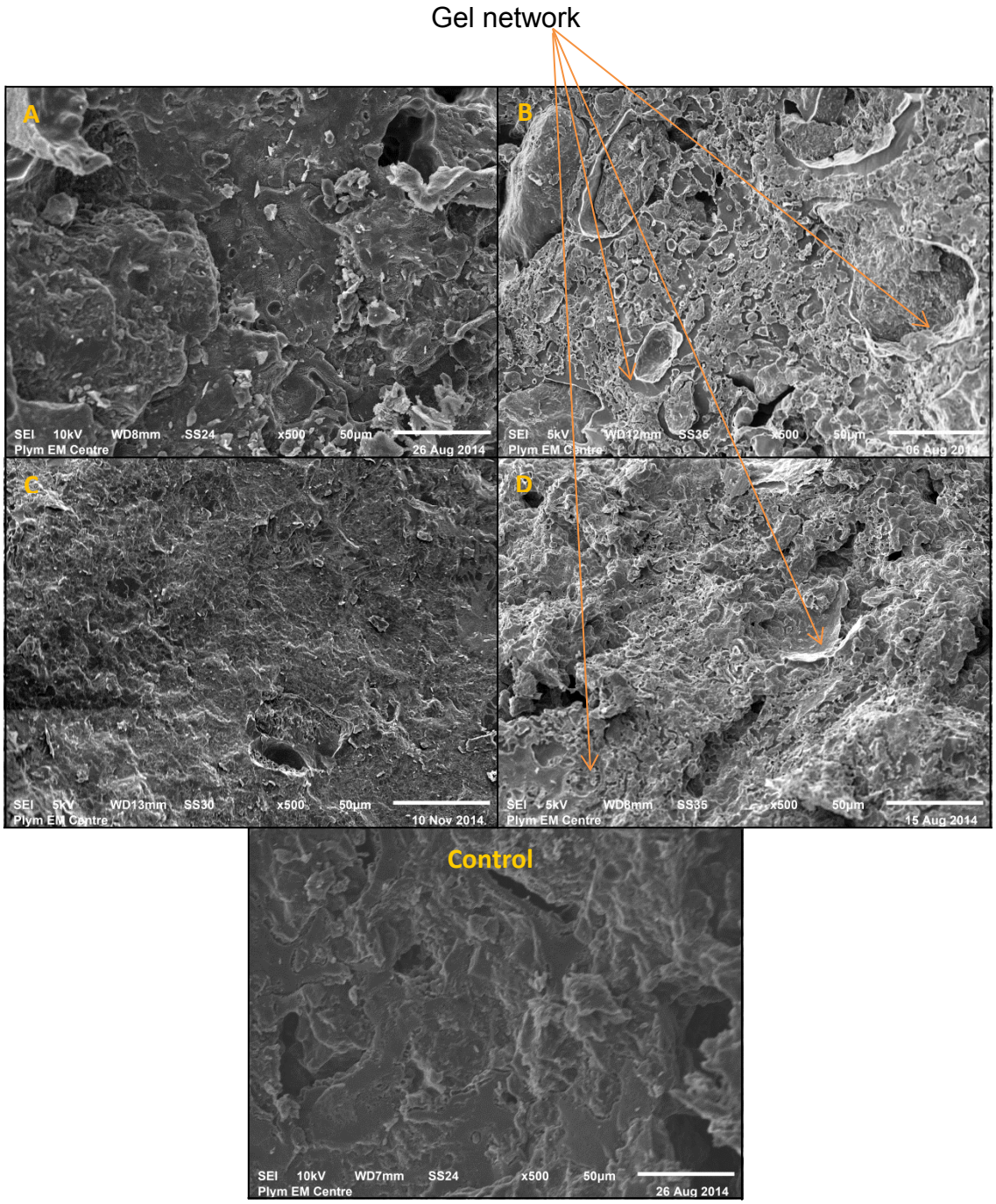


Figure 3.12: Cryo-SEM micrographs at 500 x magnifications of cooked samples, control (no inulin), A (7% JA.P), B (7% JA.G), C (7% CI.P), and D (7% CI.G)

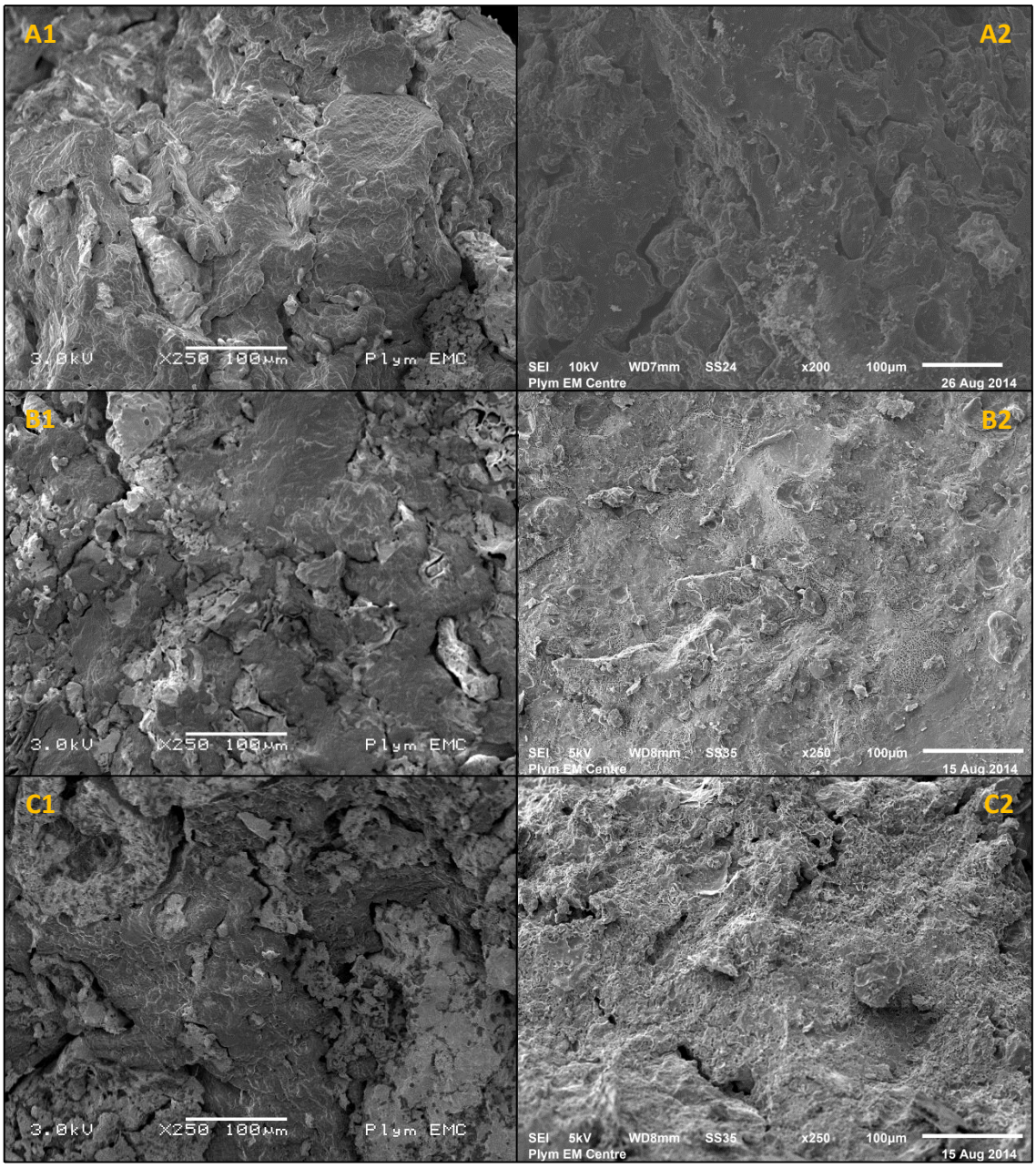


Figure 3.13: Micrographs at 250 x magnifications of cooked doner kebab samples, A1-A2 (control), B1-B2 (7% JA.G) and C1-C2 (7% CI.G) with two SEM techniques; on the left LV-SEM and on the right Cryo-SEM

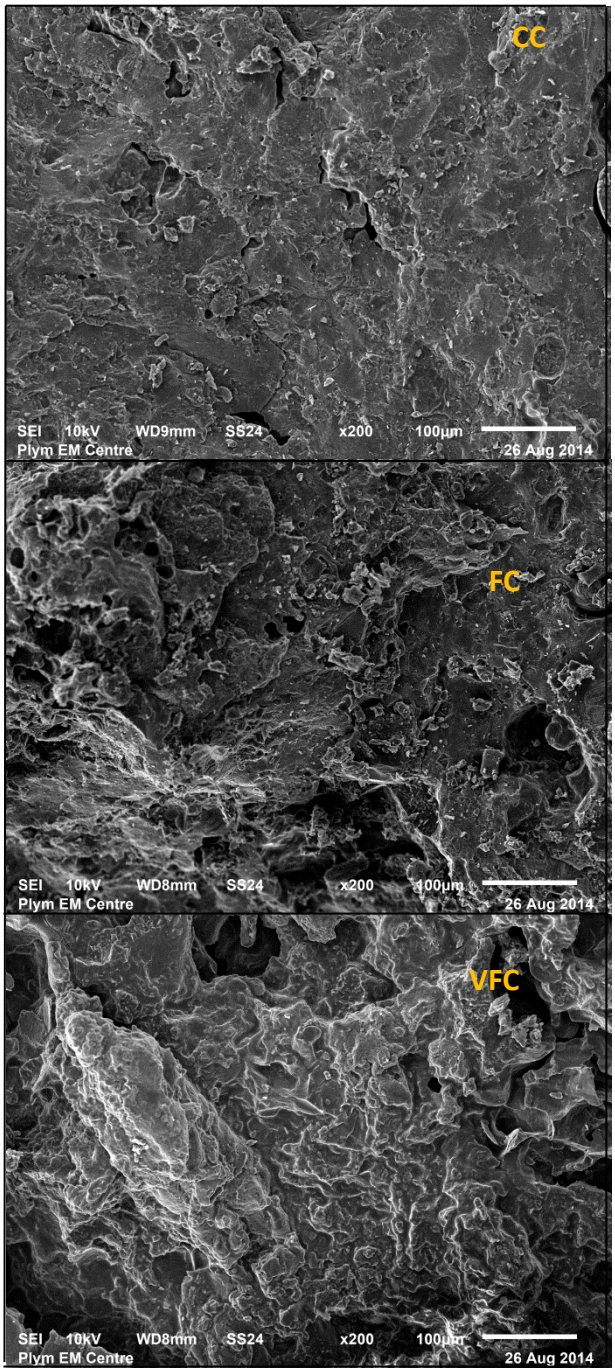


Figure 3.14: Cryo-SEM Micrographs at 200 x magnifications of cooked doner kebab samples with 7% JA.P at coarse (CC), fine (FC), and very fine chopping (VFC) levels

3.3.7 Inulin identification for authentication of labelling purposes

The aim of this investigation was to assess the usage and the accuracy of this method for determination and quantification of the inulin inclusion in meat products for authentication of labelling purposes. The Megazyme enzymatic method to measure total fructan (Muir *et al.*, 2007) based on the AOAC method 999.03 has been described as reliable for this purposes (Franck and Bosscher, 2009).

Generally, most of the quantitative methods for measuring of inulin-type fructans involve enzymatic hydrolysis of all fructan materials (Vendrell-Pascuas *et al.*, 2000), including enzymatic spectrophotometric assay applied in the present study. The general principle of the method is based on the extraction of inulin with distilled hot water, followed by hydrolysis of sucrose and low DP maltosaccharides. Then the solution was hydrolysed with fructanase enzymes, and determination of the released fructose and glucose. The difference between the content of each sugar with and without enzyme hydrolysed was the amount of fructan (most exclusively inulin) (Muir *et al.*, 2007) as described in Figure 3.6 and calculated in Appendix 3.4.

3.3.7.1 Validation and quality control of analytical method

In order to achieve sufficiently accurate results according to the assay procedure, the values of ΔA_S and ΔA_{F+S} should not be less than 0.100 absorbance units. The present values of ΔA_{F+S} were all more than 0.100, while for ΔA_S, a few samples were less than 0.100. The reference sample provided with the assay kit (fructan control flour with 27.5% inulin) was used as reference

material for checking the accuracy of the method. The obtained result of the reference sample (Table 3.10) was 27.21±0.94 with a recovery of 98.95±0.94 g/ 100g which is within the accepted range (AOAC, 2012) indicating good accuracy performance (AOAC, 2012).

The limit of detection (LoD) is about 0.2g/100g according to the assay procedure; therefore all obtained values less than 0.2g/100g in present study were considered not detectable. These values were only obtained in two treatments where no inulin was added in the prepared control doner kebab and in commercial doner kebab (Table 3.10).

3.3.7.2 Inulin determination in Jerusalem artichoke and commercial inulin

Chicory and JA are the two plant species that are most commonly used by industry to produce inulin (Nair *et al.*, 2010). They are rich in inulin in their underground parts (Simonovska, 2000) with an approximate of 15-20% in fresh bases in both plants, and around 50% of dry weight in the flour of JA (Van Loo *et al.*, 1995).

The quantified amount of inulin on the prepared JA flour was 56.62±1.43 g/100 with a moisture content of 7.56±0.30. Published papers reported different amounts of inulin identified in the prepared flour of JA tubers. The closer results to the present study of dry weight using same method (Megazyme enzymatic approach) was reported by McCleary *et al.* (2000) with a mean of 51.65g/100g, and 48.8g/100g by (Muir *et al.*, 2007).

Some other published papers reported higher values (74.48 g/100 g in dry matter bases) using the same assay on tubers of JA harvested in KRI (Akoy,

2015), and with response surface method (RSM) and thin layer chromatographic technique, 72.99±2.34% (Gaafar *et al.*, 2010), 52.5- 65.7% by Lingyun *et al.* (2007) with approximate of 5.61% moisture. Saengkanuk *et al.* (2011) quantified the inulin content in JA tubers in a range of 62.96-74.9% using a simplified spectrophotometric, while the results found by Fleming *et al.* (1979) was in the a range of 68-83% in dry weight.

These significant variations of inulin content of JA tubers are due to several reasons, including harvesting time of year and maturation, cultivar, production conditions, postharvest treatments, preparation methods (Kays and Nottingham, 2008), and storage time conditions on JA tubers (Saengthongpinit, 2005). For example, the older tissue composes less storing of fructan (Housley and Pollock, 1993). Moreover, Zubr and Pedersen (1993) pointed out that the dry matter accumulation in tubers of JA was higher in early cultivar compared to late cultivar. Kiehn and Chubey (1993) stated that due to the low rainfall and very high temperature, the yield of dry matter was reduced on JA.

Furthermore, Bornet (2008) investigated the inulin content of JA in 11 cultivars with DP over 4. The inulin content ranged from 55.8 to 77.3% (average, 65.8%) of the total carbohydrates. The DP of inulin in JA tubers ranged up to above 40 DP. Furthermore, inulin in JA has different DP content and the DP decreased as the harvesting time is delayed (Van Loo *et al.*, 1995).

Saengthongpinit (2005) studied the effect of harvesting time and maturation of JA in Thailand. It was measured that tubers of JA at 16-18 weeks contained high DP fructan (DP>10; 44.22% and 43.85%) compared to 20 weeks (40.71%) where inulin depolymerisation occurs. Therefore, early JA tubers harvested 18

weeks after planting are preferable for the production of inulin, and seemed to be optimum maturity as it had high DP fraction and low content of sucrose and fructose.

On the other hand, according to the manufacturer (Sensus, Roosendaal, Netherlands), the CI have an approximate of 96.7% of inulin content. The result obtained was only 93.29±2.55 with recovery of 96.47±2.55 (Table 3.10) which is very close to the accepted range (AOAC, 2012). One reason for this lower recovery could be the moisture absorbance while the material was stored over time or during usage. A factor that may explain this low recovery is the high moisture levels in the sample, 6.35±0.28 (Table 3.1) compared to a 3% moisture reported on the information sheet by the supplier (Sensus, Roosendaal, Netherlands).

3.3.7.3 Determination of inulin content in meat products

Inulin is classified as food or food ingredients, and not as an additive. EU directive EC 95/2 listed inulin as substance that is not an additive (Coussement, 1999). Therefore, it can be added into meat products in a range of 2-10 (w/w) (Franck, 2002), or 3-6g per portion where no symptoms of discomfort can occur (Coussement, 1999). Therefore, a proper labelling of food containing inulin will give choices to consumers in order to make an appropriate amount of daily intake (Carabin and Flamm, 1999). However, the commercial peperami snacks meat used in this study with added inulin did not specify the levels of inulin, which could mislead consumers in terms of labelling information.

Table 3.10 shows results of identified inulin, recovery, and the additional or claimed amount of inulin in all analysed samples (g/100g). The added amount of inulin in meat products in each treatment was calculated based on the identified amount of inulin in JA flour (56.62±1.43) and CI powder (93.29±2.55) g/100g. Good recovery ranges (Table 3.10) were obtained for most of the treatments. This indicates an acceptable accuracy, and the applied method could be reliable for routine analysis of quality authentication and labelling purposes.

However, this assay was not as reliable for measuring fructan in food samples when it is present at less than 0.2g/ 100g of food or meat samples in dry weight basis. For those samples with values in the range up to 0.2g/ 100g, fructan was considered “not detected”.

Although this method has not been reported for inulin determination in meat products, Vendrell-Pascuas *et al.* (2000) used HPLC with refractive index detection for determination of inulin in a range of commercial meat products with excellent precision, recovery and sensitivity.

Table 3.10: Results for determination of inulin contents in meat products and plant materials (g/ 100g)

Meats/ plant materials	Fructan (inulin) g/100g		Recovery g/100g
	Added or claimed	Found	
Fructan control flour	27.5	27.21±0.94	98.95±0.94
CI (Frutafit TEX)	96.7	93.29±2.55	93.24±2.55
JA flour	52-75	56.62±1.43	N/A
Control meat	None	0.07±0.04	N/A
0.5% JA.P	0.28	0.27±0.03	96.42±0.03
0.5% CI.P	0.48	0.47±0.07	97.92±0.07
1% JA.P	0.57	0.53±0.05	92.98±0.05
1% CI.P	0.97	0.93±0.08	95.87±0.08
2% JA.P	1.13	1.19±0.02	105.30±0.02
2% CI.P	1.93	1.87±0.13	96.89±0.13
5% JA.G	2.83	2.71±0.27	95.76±0.27
7% JA.P	3.96	3.65±0.34	92.17±0.34
7% CI.P	6.77	6.53±0.02	96.45±0.02
Original Peperami	Unknown addition	2.40±0.01	N/A
Peperami added 3% JA.P	1.69	1.64±0.04	97.04±0.4
Peperami added 3% CI.P	2.90	2.80±0.19	96.55±0.19
Commercial doner	None	0.21±0.01	N/A

Mean values ± SD of three measurements. N/A, not applicable

3.4 Conclusion

Comminuted meat products such as doner kebab are commonly high in fat content. Conscious consumers are increasingly aware of a diet rich in fat and deficient in dietary fibre including meat and meat products. The introduction of both sources of inulin had the potential to influence and improve the quality image of low fat doner kebabs, considering quality characteristics and eating properties.

Reducing 50% of the fat content and adding two sources of inulin into prepared doner kebab samples could provide not only a reduction in fat intake, but it would also contribute to an increase the fibre intake of users among the general public where intake is below recommendations.

Results show that cooking properties were dramatically improved with added inulin compared to the control. This was greater when it was added as a gel instead of powder at the same levels. JA showed better acceptability of most of the sensory attributes by testing panels. Inulin in the form of gel produced a softer and less chewy product compared to the powder, which increased hardness. Electron microphotographs also supported this evidence, as the formation of a gel network that holds moisture is thought to subsequently have contributed to an increase in moisture retention, cooking yields, and improved texture. The yield and other cooking properties in fine chopping were greater than very fine and coarse chopping.

The negative effects associated with the over-consumption of inulin could include abdominal discomfort with doses of around 30g/ day. The Megazyme enzymatic assay was used for quantitative inulin in prepared and commercial

meat products. The method was suitable for the routine quantification of fructan-inulin type in JA flour and meat products with a very good recovery.

To sum up, these results indicated the possibility of manufacturing low-fat doner kebab enriched with inulin up to 7%. This would not negatively affect overall acceptability, but would improve the quality image, besides the health benefits linked to dietary fibre. Inulin applications, especially which obtained from JA from the KRI region, could be further developed for different food products including meats, bakery and desserts. Therefore, preparing flours from JA tubers could be an appropriate technology in an area of the world where specialist food ingredients appear to be costly due to currency differences and the costs of importing goods.

Chapter 4

Fish labelling at commercial markets in Kurdistan Region- Iraq: Application of DNA barcoding for fish authentication

4.1 Introduction

Due to changes in public awareness towards health and nutrition, the consumption of seafood and derived products has increased over recent decades (Verbeke and Brunso, 2005). Fish in particular has a very positive image among consumers in terms of health (Vanhonacker *et al.*, 2013). This is mainly due to the fact that fish are widely known to be high in protein and PUFAs (Sidhu, 2003). The health benefits of fish and seafood has also been widely encouraged in media (Yadavalli and Jones, 2014).

It is currently estimated that more than 800 fish species are traded internationally under different forms, shapes, brands and preparation methods (FAO, 2004). As a result of the globalization of the seafood trade, increasing demand for seafood, but a lack of traceability, the seafood markets in many countries are highly vulnerable to fraud practices (Jacquet and Pauly, 2007). Seafood fraud as a global problem can take different forms, including false labelling, species substitution and false origin authentication (i.e. wild or farmed fish) (Golden and Warner, 2014).

Both consumers and governments can lose financially as a result of mislabelling. In addition to economic losses, there could be losses of resources and health concerns (Jacquet and Pauly, 2008). For these reasons authentication of fish and seafood products has become a crucial issue (Rasmussen and Morrissey, 2008). According to the EU directive EC/2065/2001, it is a legal requirement that the seafood sector must provide the consumer clear labelling information including species identity, geographical origin, and production method of fish (i.e. wild or farmed). Each of these factors can be an opportunity for mislabelling. It is suggested that mislabelling is most often done by distributors and the final

seafood retailers (e.g., fishmongers and restaurants). This could be because there are no regulations at catering services compared to wholesale, where regulations are applied (Jacquet and Pauly, 2008).

Whole fish identification is traditionally based on external morphological features (Strauss and Bond, 1990). However, difficulties arise when such methods are used to identify fish products, such as fillets and other processed forms, which are lacking in morphological characteristics. Moreover, in some cases the morphological features are not adequate for identification and differentiation, even with whole specimens, as they can show either considerable intraspecific variation or small differences between species (Teletchea, 2009).

Several traditional methodologies have been applied previously for authentication of different fish species based on the separation and characterization of specific proteins using electrophoretic techniques, such as IEF (Renon *et al.*, 2005), HPLC (Knuutinen and Harjula, 1998), immunoassay, such as ELISA (Taylor and Jones, 1992), or proteomics (Mazzeo and Siciliano, 2016).

As an alternative to protein analysis, many DNA-based techniques have been recently developed for identifying fish from commercial markets (Folmer *et al.*, 1994, Wolf *et al.*, 2000, Ward *et al.*, 2005, Ivanova *et al.*, 2007, Nicolè *et al.*, 2012, Mueller *et al.*, 2015). However, to select an appropriate DNA-based approach, it is important to consider a number of factors, including: availability of expertise and laboratory resources, time limitations, financial constraints, and most importantly, the research question pursued (Pereira *et al.*, 2008). Furthermore, the reliability of the technique, the range of the target species,

sampling process, and ability to recover and identify DNA from processed products should also be considered (Rasmussen and Morrissey, 2008, Hellberg and Morrissey, 2011, Galimberti *et al.*, 2013).

In recent years DNA barcoding has been proposed as the preferred methodology in forensic taxonomy, including fish identification (Ward *et al.*, 2005, Dawnay *et al.*, 2007). For example, in the last decade the FDA of the United States has implemented DNA barcoding to forensically identify fish products (Yancy *et al.*, 2008).

Generally, DNA barcoding is based on the amplification of the mtDNA COI gene, cytochrome *b* (Cytb) gene, control region (d-loop) or 16S rRNA, depending on the taxa, to act as a "barcode" for species identification (Ward *et al.*, 2005, Roe and Sperling, 2007). Numerous studies using DNA barcoding have revealed different rates of seafood fraud in several countries and with different species, where valuable fish are substituted and/or mislabelled with those of lower value (Filonzi *et al.*, 2010, Hanner *et al.*, 2011, Cawthorn *et al.*, 2012, Cline, 2012, Galal-Khallaf *et al.*, 2014, Carvalho *et al.*, 2015b, Lamendin *et al.*, 2015, Chang *et al.*, 2016, Chin *et al.*, 2016, Nagalakshmi *et al.*, 2016, Yan *et al.*, 2016). This is clear evidence that mislabelling has been recognized internationally as a significant issue (Miller *et al.*, 2012).

Lack of regulation and labelling enforcement is one of the main contributors to seafood mislabelling (Miller and Mariani, 2010, Hanner *et al.*, 2011), and this can happen at different points in the supply chain (Cawthorn *et al.*, 2013). However, not all mislabelling may be deliberate, and it is often the case that the same fish names are applied to different species unintentionally in different

regions, and due to the lack of specific denominations, especially for new exotic species (Armani *et al.*, 2012, Lamendin *et al.*, 2015). Therefore, seafood trading must require boat-plate traceability for wild and farmed seafood to combat this global problem and verify the legality of products in the supply chain while providing consumers with more information about the seafood they eat (Golden and Warner, 2014).

To date, there have been no published reports on the use of DNA barcoding to confirm the species identity and to assess the accuracy of fish labelling at commercial markets in KRI. The present study aimed to assess label truthfulness of a variety of fish species commercially traded in KRI using DNA barcoding with the Cytb and/or COI genes.

The specific objectives were to:

- Use DNA barcoding to confirm the identity of different fish species bought in Kurdistan markets and to detect potential mislabelling issues.
- Determine whether DNA barcoding can be used to distinguish between wild and farmed common carp.

4.2 Materials and methods

4.2.1 Sample collection

A total of 120 samples of ten different fish species, comprising 87 local carp (Cyprinidae) and 33 imported fish [(whole fresh, filleted (fresh and frozen), and cooked)] were bought from different markets in Erbil province, KRI between December 2014 and January 2015 (Table 4.4 and Appendix 4.1, 4.2).

Samples were transported in an ice box and stored at 4°C until further processing. Small pieces of dorsal muscle tissue (1 to 1.5g) were cut from each fish sample and preserved in a cryo-tube filled with absolute ethanol. Prior to shipping the samples to the UK, the ethanol was decanted off the tissues, leaving the specimen only moist. After an approximate of 24 h shipping, absolute ethanol was added immediately on arrival at the laboratory at Plymouth University, UK, and stored at room temperature before further analysis.

4.2.2 DNA extraction, amplification and sequencing

Total genomic DNA was extracted from approximately 20mg of dorsal muscle tissue following a rapid and inexpensive HotSHOT method (Truett *et al.*, 2000), and then stored at 4°C (short time) and -18°C (long term) before further analysis. The DNA concentration (ng/µl) of each sample was determined by using NanoDrop® ND-1000 spectrophotometer (Thermo Scientific Nano Drop™ 2000, DE, USA), (the DNA concentrations of all samples were ≥ 20 ng/µl), and then all were diluted with molecular grade water to be standardized them to 15 ng/µl.

Originally the COI region was amplified using standard Folmer primers (Folmer *et al.*, 1994), but most of the tested samples failed to amplify even after several optimization attempts. Instead, specific fish barcoding primers developed by Ivanova *et al.* (2007) (Table 4.1) were tailed, but while successful, these resulted in non-specific PCR products for several samples.

Therefore, the Cytb region was used as the premier barcoding method and the COI region was used where this failed to amplify and to check ambiguous identifications. PCR was performed in a Prime Thermal Cycler (Bibby Scientific Ltd, UK) at Plymouth University, molecular ecology laboratory, UK. A pair of primers (Table 4.1) reported by Wolf *et al.* (2000) were used for PCR amplification of a 464 bp fragment of the mitochondrial Cytb gene. Each PCR tube contained a total of 25 µl for each sample (Table 4.2). Gradient PCR was performed initially to optimize the annealing temperature and from this optimum temperature was found to be 53°C (see Table 4.3 for full PCR cycling conditions).

For the COI PCR, primers FishF2_t1 and FishR2_t1 (Ivanova *et al.*, 2007) were used to amplify a partial 655bp region for a total of 23 samples (Table 4.5),

consisting of those samples that failed in the Cytb PCR (16 samples) and another seven samples from the carp family that were randomly selected from those already identified by Cytb. In this method trehalose was used for PCR amplification as a PCR enhancer in order to retain higher concentrations of DNA, reduce DNA melting temperature, thermostabilise *Taq* DNA polymerase and recover higher amplification yield (Spiess *et al.*, 2004). The PCR mixtures and cycling conditions are given in Table 4.2 and 4.3 respectively. The PCR products for all samples were stored in the fridge (short-term use) for further analyses.

The quality of PCR products was checked by gel electrophoresis on a 2% agarose gel stained with 5 µl of SYBR® Safe DNA Gel stain (Thermo Fisher Scientific, Paisely, UK) at 100V for one hour, and imaged on an ImageQuant LAS4000 (GE Healthcare Life Science, UK). There were clear PCR products for approximate 464 bp fragments of the Cytb gene (Figure 4.1), while some non-specific bands for the COI region (Figure 4.2) were found, and it is therefore the Cytb marker was used as the premier method for most of the samples.

Amplified PCR products were cleaned to remove excess primers and dNTPs using 10 Units of Exonuclease I and 1 Unit of FastSap (Amersham Bioscience, Buckinghamshire, UK) per 20 µl of PCR product and these were then sequenced by Macrogen Inc., Netherlands, using the primers H15149ad (Cytb) or FishF2_t1 (COI).

Table 4.1: Primers used for PCR amplification and sequencing

Region amplified	Primer name	Primer sequence (5'-3')	No. of bases	Fragment size/bp	samples amplified	Reference
Cytb	H15149	5'-GC**H**CCTCA**R**AATGA**Y**ATTTGTCCTCA	26	~464	104	(Wolf *et al.*, 2000)
	L14735	5'-AAAAACCACCGTTGTTATTCAACTA	25			
COI	FishF1_t1	5'-TGTAAAACGACGGCCAGTCGACT AATCATAAAGATATCGGCAC	43	~655	23	(Ivanova *et al.*, 2007)
	FishR1_t1	5'-CAGGAAACAGCTATGACACTTCAG GGTGACCGAAGAATCAGAA	43			

Table 4.2: Reagents used for PCR amplification for each sample

Reagents	**DNA sequencing with Cytb gene**		**DNA sequencing with COI gene**	
	Concentration	Volume (µl)	Concentration	Volume (µl)
Template DNA	15ng/ µl	1	15ng/ µl	2
Forward primer	15mM	2	10mM	0.125
Reverse primer	15mM	0.5	10mM	0.125
BioMix^{TM}	10X	12.5	---	6.25
Molecular H_2O	---	9	---	---
Trehalose	---	---	10%	4
Total	**---**	**25**	**---**	**12.5**

Table 4.3: PCR conditions for the different target genes

Step	**Cytb**	**COI**
Initial denaturation (°C/min)	95/5	94/2
Denaturation (°C/s)	95/40	94/30
Annealing (°C/s)	53/80	52/40
Extension (°C/s)	72/80	72/60
Final extension (°C/min)	72/7	72/10
Number of cycles	35	35

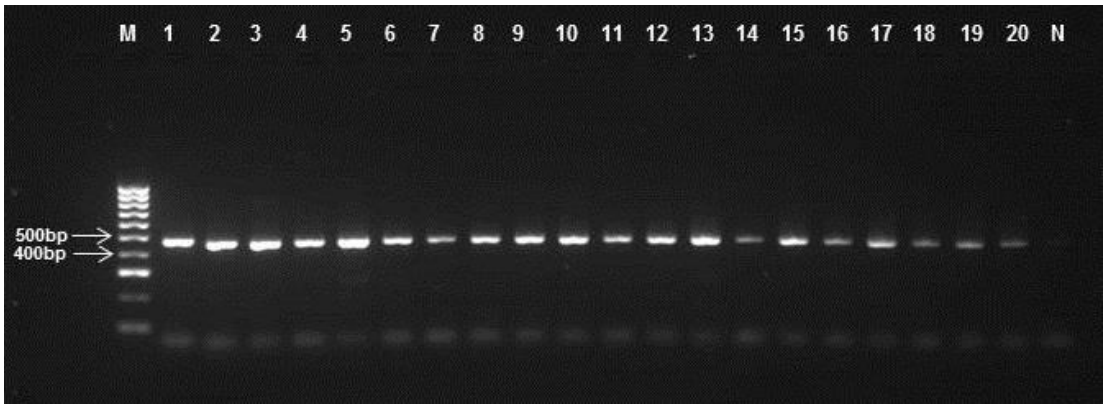


Figure 4.1: Agarose gel of PCR products from the Cytb gene of fish samples bought from the markets. M= ladder (100bp); Lanes 1-20= fish samples of various species; N= negative control

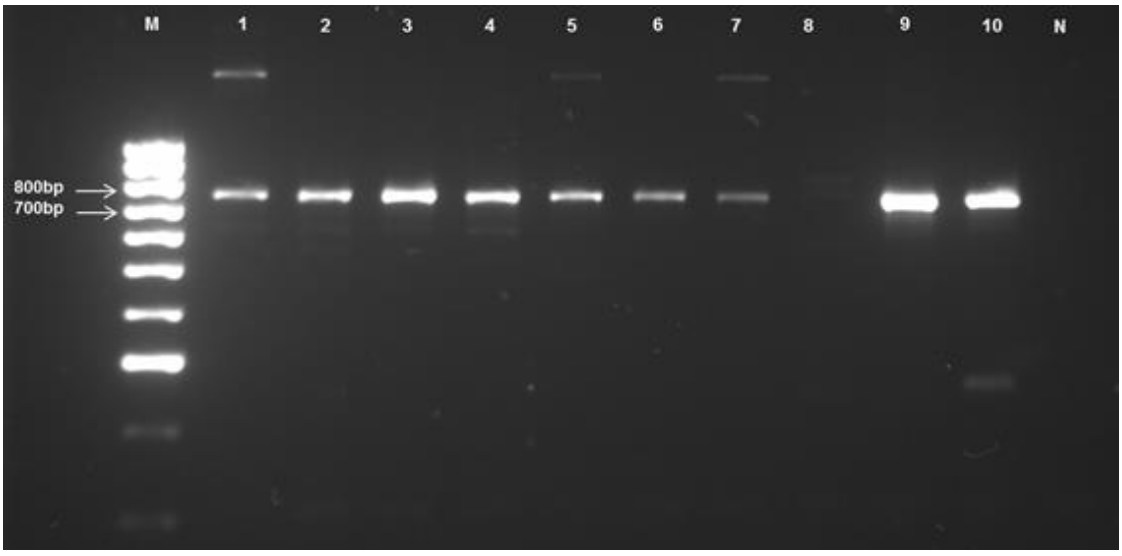


Figure 4.2: Agarose gel of PCR products from the COI gene of fish samples bought from the markets. M= ladder (100bp); Lanes 1-10= fish samples; N= negative control

4.2.3 Sequencing data processing

DNA sequences were manually edited using Bio-Edit software version 7.2.5 (Hall, 1999) to trim the sequence ends. Sequences were checked for quality by translating nucleotide sequences into a protein sequence using the EXPASY translate tool (SIB Web Team, 2011, http://web.expasy.org/translate/) to ensure the sequences adhered to an open reading frame. Only 6 samples out of 120 had stop codons from Cytb gene (88-SB, 113-MA, 117-CF, 118-CF, 119-CF, and 120-CF), and they were excluded from the analysis, because they contained underlying sequence.

These sequences were then compared with reference sequences using BLAST (Basic Local Alignment Search Tool) (Altschul *et al.*, 1990) searches against GenBank (NCBI, https://blast.ncbi.nlm.nih.gov/Blast.cgi) and/or the Barcode of Life Database system (BOLD System V3) (for COI sequences only). As a general rule, a top match with a sequence similarity of 98% or more was used to assign an identity to the samples collected (Table 4.4 and 4.5). The reason for considering a match of less than 98% as unreliable match is associated with several published papers which also used a top match with a sequence similarity of at least 98% to designate potential species identification (Barbuto *et al.*, 2010, Cawthorn *et al.*, 2012, Armani *et al.*, 2015).

4.2.4 Haplotype analysis and phylogenetic trees

Pairwise *p*-distance was calculated using MEGA 6 (Tamura *et al.*, 2013) to assess the intraspecific genetic distance between individuals. The number of haplotypes (Hap) was calculated for all species with more than four samples using DnaSP v.5 (Librado and Rozas, 2009), following the removal of sites containing gaps/ missing data. One sequence of each haplotype was used in subsequent analysis.

From 53 *Cyprinus carpio* individuals, eight haplotypes were identified (haplotype 1, 29 individuals; haplotype 2, 12 individuals; haplotype 3, 7 individuals; haplotype 4, 5, 6, 7, and 8 each 1 individual). *Carassius auratus* consisted of 9 individuals belonging to four haplotypes (haplotype 1, 1 individual; haplotype 2, 2 individuals, haplotype 3, 1 individual; haplotype 4, 5 individuals). *Capoeta barroisi* consisted of 14 samples belonging to four haplotypes (haplotype 1, 1 individual; haplotype 2, 8 individuals; haplotype 3, 1 individual; haplotype 4, 4 individuals). From 8 individuals of *Salmo salar*, three haplotypes were identified (haplotype 1, 6 individuals; haplotype 2, 1 individual; haplotype 3, 1 individual), while from 9 samples of *Arabibarbus grypus* only two haplotypes were determined (haplotype 1, 8 individuals; haplotype 2, 1 individual).

Sequences were aligned using Clustal W alignment in MEGA 6 (Thompson *et al.*, 1994) together with sequences obtained from GenBank as validated references for each species for both regions and given with accession numbers (Figure 4.4 and 4.5). Due to the short sequences of some samples, an aligned region of 333 bp was used for comparison for the Cytb gene and a 559 bp region was used for the COI gene in the final analysis.

Three types of phylogenetic trees were constructed in MEGA 6 for Cytb and COI separately using Neighbour Joining (NJ) (Saitou and Nei, 1987), maximum likelihood (ML) and minimum evolution (ME) to determine the likely phylogenetic relationships for all species with reference sequences retrieved from Genbank with *P*-distance as a measure of genetic distance for both NJ and ME trees, according to the recommendations of Nei and Kumar (2000), while for ML tree, the Kimura 2-parameters model was used instead of *p*-distance (because only this method was available) (see Appendix 4.3 for ML and ME trees).

NJ, ML, and ME are just three of a number of methods used for phylogenetic inference. It has been suggested that NJ should only be used as a starting tree for further search using branch swapping under ME tree criteria (Swofford *et al.*, 1996), but selecting a method depends upon a set of criteria including efficiency, robustness and computational speed, as well as the data under study (Hall, 2004).

To assess the reliability of the trees, bootstrap analysis (Felsenstein, 1985) was performed using 1000 replicates to verify the robustness of the phylogenetic relationships. All positions containing gaps and missing data were eliminated by choosing the complete deletion option. The transitions and transversions substitution and homogenous pattern among lineages were chosen with uniform rates among sites.

4.3 Results and discussion

4.3.1 Species identification

In recent years, attention has been turning towards DNA-based approaches for fish species authentication (Asensio *et al.*, 2009), and DNA barcoding has proven to be a successful species identification tool for whole, filleted, raw and cooked fish as well as any other properly preserved tissue (Hanner *et al.*, 2011, Lamendin *et al.*, 2015).

During the sample collection in the present study, lack of enforcement regulations on fish labelling were observed at selling points in the KRI, with some cases of ambiguous naming of fish species, especially in fishmongers and street markets. About 90% of collected fish samples were described with the local name (common name), while 10% were only declared as wild unknown species (WUS). The declaration of fish origin (wild or farmed) was also ambiguous, and carp samples were often sold without a species name. However, supermarkets that sell local and imported species tend to have better labelling information with local naming, but this ambiguity is potentially concerning for consumers of commercial markets in KRI.

In the present study, a total of 104 (87%) out of 120 samples were successfully identified with the Cytb gene. The results demonstrated unambiguous species identification (Table 4.4) with high matches (≥98%) to sequences available in the GenBank database, suggesting this is a suitable technique for the genetic identification of most fish species where there is an existing reference sequence available (Perez and Presa, 2008, Lago *et al.*, 2013).

Of the 16 unidentified samples at this region, only two specimens (58-WCC and 114-NP) did not yield any useable sequences matched with BLAST to any species identity as their sequences have no signal. Another 14 sequences did not yield BLAST hits with sufficiently high matches (at a threshold of 98%) for species identification (only between 85-97%). Therefore, these samples (16) were eliminated from subsequent data analysis for Cytb sequences.

The 23 samples listed in Table 4.5 (14 which yielded low BLAST matches at the Cytb region, two specimens that did not yield any usable sequences and seven randomly chosen carp samples that were barcoded at the Cytb region) were successfully identified with COI region to the species level in both BLAST and BOLD databases (Table 4.5), and in all cases they correctly matched the declared species (similarity ≥98). The DNA sequences ranged from 637 to 785 base pairs (mean= 696 bp) with no stop codons detected.

This may reflect the fact that the COI region is more widely used and that there were reference sequences available for more fish species at this region, which is a positive reason to use this region over the Cytb region for species identification in these species. However, due to the non-specific products found during the COI amplification, in this study this region was not used as the premier barcoding method.

Some observations were made when sequences (23 samples) of the present study were compared either to BOLD and GenBank. For example, barcode data from BOLD yielded superior species matching in comparison to those achieved in GenBank with slightly greater similarity matching (Table 4.4 and 4.5).

However, three samples of *Arabibarbus grypus* (79-SHB, 84-SHB, 87-SHB) were not assigned to any species by the BOLD search engine as there was no COI barcode sequence available for this species in the BOLD database (Table 4.5). In contrast, these three samples were identified and matched to correct species (*Arabibarbus grypus*) with BLAST either with Cytb and COI sequences.

The three samples sold as kingfish (108-KF, 109-KF, and 110-KF; Table 4.4) were identified as *Scomberomorus commerson* with Cytb primers when compared with BLAST (94-96% match), which matched the declared label. However, this match was considered to be insufficient, because matches with less than 98% similarity were below the threshold of reliability. Therefore, the three samples were additionally sequenced with COI primers for reassurance. The identified species were confirmed to be of the same species (*Scomberomorus commerson)* with similarity of 99-100% both in BLAST and BOLD (Table 4.5). This suggests Cytb could be more variable for some species and a lower threshold could be used.

Another three samples declared as Sultan Ibrahim, Nile Perch and Cuttlefish (107-SI, 116-NP, and 118-CF) respectively had an unreliable match, with a similarity of 97% in BLAST with their Cytb sequences (Table 4.4). The identified species of the two samples (116-NP, and 118-CF) was *Cyprinus carpio,* but they were sold as Nile Perch and Cuttlefish respectively, while the other sample (107-SI) was matched as *Carassius auratus*, but declared as Sultan Ibrahim (Table 4.4). But, when these samples were sequenced with the COI gene, all three were correctly matched to the declared species with similarity of 99% in both BLAST and BOLD (Table 4.5). This could suggest that there was an error in labelling of these samples during the Cytb PCR and/or sequencing process,

but this may also reflect the quality of data available in the BLAST and BOLD databases.

In general, samples identified with COI had higher match with those in reference databases compared to the Cytb region. For example, all 23 samples sequenced with the COI gene had a high match of more than 98% with both GenBank and BOLD, while only 104 (87%) out of 120 samples were identified with Cytb gene at same level of similarity. Therefore, the COI gene could be a better region to use for these species identification.

For example, BOLD has a stricter submission process which requires detailed information on the taxonomy, including morphological identification, specimen details and collection information. GenBank does not require such information, though it can be added, and so it is harder to verify records and possibly leads to erroneous species identifications. This suggests that the species studied, barcoding regions used, and reliability of reference sequences should be carefully considered when using DNA barcoding for authentication.

However, when PCR products of both methods were checked with agarose gel, COI marker had some non-specific bands compared with a clear PCR products with Cytb gene (Figure 4.1 and 4.2), and due to more difficulty/ costly to process COI gene on a large scale, Cytb gene was used as premium method.

Phylogenetic trees are often constructed to find the genetic relationships between various organisms based on the information extracted from genetic material such as DNA sequences (Fitch and Margoliash, 1967). Here, the phylogenetic trees were constructed with obtained sequences and validated reference sequences retrieved from GenBank to assess and confirm the genetic

relationships within and between species, and between wild and farmed common carp (Figure 4.3 and 4.4).

All the specimens were clustered in agreement with their genetic identification at the species level with a high bootstrap support values, together with the top matched reference sequences from GenBank, giving an indication of the robustness of the tested methods (Fig 4.3 and 4.4). Furthermore, no taxonomic deviation was detected at the species level. The genetic distance between the obtained sequences of this study and the reference sequence of the database with sample species was zero for most species, meaning they belong to the same haplotype for this length of sequence (333bp Cytb, 559bp COI) (Appendix 4.4A, and 4.4B).

The NJ (Figure 4.3) and other two trees (ML and ME) (Appendix 4.3) were similar in terms of grouping same species with each other. From each species one reference sequence was used, and they were clustered with the obtained reference with high bootstrap support. Using reference sequences from public databases may add the analysis method more robustness.

4.3 2 Assessment of fish mislabelling at commercial markets

To date, research on authentication of commercial fish species and its origin for labelling purposes is rarely reported in Iraq and so is difficult to compare with the present study; most of the published papers on fish identification focused on identification of the Iraqi fish fauna rather than fish authentication. For example, Faddagh *et al.* (2012) investigated DNA fingerprinting of eight Cyprinidae fish species in Iraq using RAPD-PCR techniques to distinguish between different types of fish species such as *Cyprinus carpio* and *Arabibarbus grypus.*

Furthermore, authentication of the most widely distributed Cyprinidae fish species has rarely been investigated. Chen *et al.* (2012) used PCR-RFLP method in Taiwan for the authentication of Cyprinidae fish species including the *Cyprinus carpio* and *Carasisu auratus*. Results showed that six out of eighteen processed samples were labelled as Cyprinidae species, but they were identified as *Oreochromis* species. In contrast, a study using PCR- RFLP reported that in the same country, milkfish was adulterated with cheaper carp species such as bighead (Chen *et al.*, 2009).

In the present study, potential mislabelling was only detected on the whole fresh samples acquired from fishmongers and street markets that were sold as wild common carp, while no mislabelling cases occurred among all the tested samples obtained from the supermarkets (33 samples), which had slightly better labelling of their fish species (Appendix 4.2). Therefore, selling points of fish species may also contribute to the potential mislabelling level.

Here, Cytb barcoding found that 12 out of 120 samples (10%) were incorrectly labelled. Among those mislabelled samples, nine samples (44-WCC, 45-WCC, 46-WCC, 47-WCC, 59-WCC, 60-WCC, 61-WCC, 62-WCC, and 66-WCC) were

identified as goldfish (*Carassius auratus)* and three samples (63-WCC, 64-WCC, and 65-WCC) as *Capoeta barroisi* (Table 4.4). One possible reason for this mislabelling particularly for goldfish samples is the similarity in morphology between common carp and goldfish, and it could be difficult for customers and retailers to differentiate them visually. Goldfish (*Carassius auratus*) is closely related to common carp (*Cyprinus carpio*) as it has the same number of chromosomes (Ojima *et al.*, 1966), and can form naturally occurring hybrids (Taylor and Mahon, 1977).

In this study, phylogenetic trees (Figure 4.3 and Appendix 4.3) placed common carp and goldfish in a separate sister clade with 63% bootstrap support, but as most closer species to common carp with relatively low genetic distance (0.07) between them (Appendix 4.4A).

The other three mislabelled samples (63-DWCC, 64-DWCC, and 65-DWCC) that were identified as *capoeta barroisi,* are not similar to common carp in morphological appearance. Mislabelling of seafood might be intentional or unintentional by fishmongers or street markets. However, in the present study it was observed that this potential mislabelling could be because fish-sellers lack the knowledge to accurately identify them based on morphological characteristics, and therefore this mislabelling could be a case of accidental rather than deliberate fraud.

The level of mislabelling (10%) in the present study using DNA barcoding tools is relatively lower compared to that found in other countries worldwide e.g. Egypt (33.3%; (Galal-Khallaf *et al.*, 2014), North America (25%; (Wong and Hanner, 2008), India (22%; (Nagalakshmi *et al.*, 2016), Brazil (24% (Carvalho *et*

al., 2015b), Malaysia (16%; (Chin *et al.*, 2016), South Africa (31%; (Cawthorn *et al.*, 2012), Italy (32%; (Filonzi *et al.*, 2010), but higher than in the UK (5.66%; (Helyar *et al.*, 2014). However, mislabelling together with other unfair practices such as intentional substitution, tampering, or mispresentation made for economic gain, is still classed as economically motivated adulteration or food fraud (Spink and Moyer, 2011).

Strong competition among retailers to protect their brands with proper labelling systems often increases transparency for consumers and leads to the higher quality observed in the large retailers (He *et al.*, 2013). However, increasing consumer awareness about mislabelling of seafood products may also raise demand for authentic food from the market (Wong and Hanner, 2008, Warner *et al.*, 2013). A study conducted in New York by Oceana on a total of 142 collected samples revealed that small markets had significantly higher fraud (40%) compared to national chain grocery stores (12%) (Warner *et al.*, 2012).

Another study (Pardo *et al.*, 2016) reviewed a total of 51 peer-reviewed papers that included 4500 analysed samples using the DNA methodologies for fish authentication. The results show that the average percentage of fish incidents was 30%, and incidents in restaurant and takeaway were much more common compared to supermarkets and retailers.

The differences in fish price and preferences for wild and farmed common carp could be another reason for this mislabelling, because all mislabelled samples were sold as wild. Wild common carp samples purchased for this study had higher price compared to farmed common carp (Table 4.4). In general, wild fish species often have premium prices over farmed fish (Bell *et al.*, 2007, Morrison

et al., 2007, Arechavala‐Lopez *et al.*, 2013), and therefore financial incentives are the strongest motivation to rename fish with more appetizing titles or mislabel seafood as a high-priced species.

For example, many fish are given an entirely new name (often similar to that of an already popular fish) to boost sales (Jacquet and Pauly, 2008). Also, fish may be labelled correctly with species identity, but hide the fact that it has been farmed (Jacquet and Pauly, 2008). For example, the FSA survey in the UK investigated whether fish described as wild was actually wild, using a novel food authenticity method developed by the agency. It was found that, of 128 fish purchased from supermarkets, fishmongers, fish auctions, and special food shops, approximately 10% of the wild sea bass, 11% of wild sea bream and 15% of the wild salmon were found to be farmed (FSA, 2007).

Wild and farmed common carp origin could not be discriminated in the present study using DNA barcoding on the basis on their position in the phylogenetic trees (Figure 4.3), and so the question remains as to whether farmed fish is being sold as wild fish for economic gain in this region.

However, other genetic technique such as microsatellite markers have been successfully used to discriminate between wild and farmed of several fish species, such as *Cyprinus carpio* (Kohlmann *et al.*, 2005), the European population of the gilthead sea bream (*Sparus aurata*) (Alarcón *et al.*, 2004), bream (*Abramis brama orientalis; berg, 1905*) (Hosseinnia *et al.*, 2014), and *sparus aurata* (Karaiskou *et al.*, 2009). For discriminating wild and farmed common carp, a separate study was carried out explained in chapter five using applications of multi-element fingerprinting and near-infrared spectroscopy for the authentication origin of wild and farm common carp (*Cyprinus carpio)*.

Table 4.4: Detailed description of all samples analysed at the Cytb region (n=120) with their declared names matched to identification results from GenBank

Sample code[1]	Fish sold as (local name)	Processing state	Sample location[2]	Price GBP£/kg	GenBank			Mislabelling?
					Species identified	Similarity (%)	Sequence length (bp)	
1- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	100	387	No
2- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	99	391	No
3- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	100	377	No
4- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	100	381	No
5- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	100	373	No
6- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	99	391	No
7- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	99	400	No
8- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	99	396	No
9- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	100	376	No
10- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	99	379	No
11- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	100	373	No
12- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	99	390	No
13- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	99	386	No
14- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	99	393	No
15- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	100	395	No
16- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	98	393	No
17- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	100	395	No
18- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	100	395	No
19- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	99	399	No
20- FCC	Common carp	Fresh whole	DF	4-5	*Cyprinus carpio*	99	396	No
21- FCC	Common carp	Fresh whole	FM	4-5	*Cyprinus carpio*	99	388	No
22- FCC	Common carp	Fresh whole	FM	4-5	*Cyprinus carpio*	99	362	No
23- FCC	Common carp	Fresh whole	FM	4-5	*Cyprinus carpio*	100	389	No
24- FCC	Common carp	Fresh whole	FM	4-5	*Cyprinus carpio*	100	383	No
25- FCC	Common carp	Fresh whole	FM	4-5	*Cyprinus carpio*	99	378	No
26- FCC	Common carp	Fresh whole	FM	4-5	*Cyprinus carpio*	100	376	No
27- FCC	Common carp	Fresh whole	FM	4-5	*Cyprinus carpio*	100	393	No
28- FCC	Common carp	Cooked	RE	8-10	*Cyprinus carpio*	100	362	No
29- FCC	Common carp	Cooked	RE	8-10	*Cyprinus carpio*	99	386	No
30- FCC	Common carp	Cooked	RE	8-10	*Cyprinus carpio*	100	371	No
31- FCC	Common carp	Cooked	RE	8-10	*Cyprinus carpio*	100	397	No
32- FCC	Common carp	Cooked	RE	8-10	*Cyprinus carpio*	100	377	No
33- FCC	Common carp	Cooked	RE	8-10	*Cyprinus carpio*	100	397	No
34- FCC	Common carp	Cooked	RE	8-10	*Cyprinus carpio*	100	396	No
35- FCC	Common carp	Cooked	RE	8-10	*Cyprinus carpio*	100	395	No
36- WCC	Common carp	Fresh whole	FM	6-7	*Cyprinus carpio*	100	374	No
37- WCC	Common carp	Fresh whole	FM	6-7	*Cyprinus carpio*	100	360	No
38- WCC	Common carp	Fresh whole	FM	6-7	*Cyprinus carpio*	100	394	No
39- WCC	Common carp	Fresh whole	FM	6-7	*Cyprinus carpio*	99	381	No
40- WCC	Common carp	Fresh whole	FM	6-7	*Cyprinus carpio*	100	400	No

Table 4.4 (continued)

Sample code[1]	Fish sold as (local name)	Processing state	Sample location[2]	Price GBP£/kg	GenBank			Mislabelling?
					Species identified	Similarity (%)	Sequence length (bp)	
41- WCC	Common carp	Fresh whole	FM	6-7	*Cyprinus carpio*	99	401	No
42- WCC	Common carp	Fresh whole	FM	6-7	*Cyprinus carpio*	100	399	No
43- WCC	Common carp	Fresh whole	FM	6-7	*Cyprinus carpio*	100	396	No
44- WCC	Common carp	Fresh whole	FM	6-7	*Carassius auratus*	99	360	Yes
45- WCC	Common Carp	Fresh whole	FM	6-7	*Carassius auratus*	99	386	Yes
46- WCC	Common Carp	Fresh whole	FM	6-7	*Carassius auratus*	99	387	Yes
47- WCC	Common Carp	Fresh whole	FM	6-7	*Carassius auratus*	100	387	Yes
48- WCC	Common Carp	Fresh whole	FM	6-7	*Cyprinus carpio*	99	398	No
49- WCC	Common Carp	Fresh whole	FM	6-7	*Cyprinus carpio*	98	393	No
50- WCC	Common Carp	Fresh whole	FM	6-7	*Cyprinus carpio*	100	393	No
51- WCC	Common Carp	Fresh whole	FM	6-7	*Cyprinus carpio*	99	393	No
52- WCC	Common Carp	Fresh whole	FM	6-7	*Cyprinus carpio*	100	397	No
53- WCC	Common Carp	Fresh whole	FM	6-7	*Cyprinus carpio*	99	367	No
54- WCC	Common Carp	Fresh whole	FM	6-7	*Cyprinus carpio*	100	372	No
55- WCC	Common Carp	Fresh whole	FM	6-7	*Cyprinus carpio*	100	396	No
56- WCC	Common Carp	Fresh whole	FM	6-7	*Cyprinus carpio*	99	392	No
57- WCC	Common Carp	Fresh whole	FM	6-7	*Cyprinus carpio*	99	397	No
58- WCC	Common Carp	Fresh whole	FM	6-7	Failed to amplify	---	---	---
59- WCC	Common Carp	Fresh whole	SM	6-7	*Carassius auratus*	99	385	Yes
60- WCC	Common Carp	Fresh whole	SM	6-7	*Carassius auratus*	99	382	Yes
61- WCC	Common Carp	Fresh whole	SM	6-7	*Carassius auratus*	99	392	Yes
62- WCC	Common Carp	Fresh whole	SM	6-7	*Carassius auratus*	99	391	Yes
63- WCC	Common carp	Fresh whole	SM	6-7	*Capoeta barroisi*	99	385	Yes
64- WCC	Common carp	Fresh whole	SM	6-7	*Capoeta barroisi*	99	366	Yes
65- WCC	Common carp	Fresh whole	SM	6-7	*Capoeta barroisi*	99	383	Yes
66- WCC	Common carp	Fresh whole	SM	6-7	*Carassius auratus*	99	387	Yes
67- WUS	Wild unknown	Fresh whole	FM	3-4	*Capoeta barroisi*	99	388	N/A
68- WUS	Wild unknown	Fresh whole	FM	3-4	*Capoeta barroisi*	100	375	N/A
69- WUS	Wild unknown	Fresh whole	FM	3-4	*Capoeta barroisi*	100	391	N/A
70- WUS	Wild unknown	Fresh whole	FM	3-4	*Capoeta barroisi*	99	370	N/A
71- WUS	Wild unknown	Fresh whole	SM	3-4	*Capoeta barroisi*	100	367	N/A
72- WUS	Wild unknown	Fresh whole	SM	3-4	*Capoeta barroisi*	99	370	N/A
73- WUS	Wild unknown	Fresh whole	SM	3-4	*Capoeta barroisi*	100	367	N/A
74- WUS	Wild unknown	Fresh whole	SM	3-4	*Capoeta barroisi*	99	380	N/A
75- WUS	Wild unknown	Fresh whole	SM	3-4	*Leuciscus Lepidus*	100	387	N/A
76- WUS	Wild unknown	Fresh whole	SM	3-4	*Capoeta barroisi*	99	387	N/A
77- WUS	Wild unknown	Fresh whole	SM	3-4	*Capoeta barroisi*	99	362	N/A
78- WUS	Wild unknown	Fresh whole	SM	3-4	*Capoeta barroisi*	99	368	N/A
79- SH	Shabbout	Fresh whole	FM	8-10	*Arabibarbus grypus*	100	376	No
80- SH	Shabbout	Fresh whole	FM	8-10	*Arabibarbus grypus*	100	373	No
81- SH	Shabbout	Fresh whole	FM	8-10	*Arabibarbus grypus*	100	374	No
82- SH	Shabbout	Fresh whole	FM	8-10	*Arabibarbus grypus*	100	385	No
83- SH	Shabbout	Fresh whole	FM	8-10	*Arabibarbus grypus*	100	377	No
84- SH	Shabbout	Fresh whole	FM	8-10	*Arabibarbus grypus*	99	368	No
85- SH	Shabbout	Fresh whole	FM	8-10	*Arabibarbus grypus*	100	397	No
86- SH	Shabbout	Fresh whole	FM	8-10	*Arabibarbus grypus*	100	361	No
87- SH	Shabbout	Fresh whole	FM	8-10	*Arabibarbus grypus*	100	376	No

Table 4.4 (continued)

Sample code[1]	Fish sold as (local name)	Processing state	Sample location[2]	Price GBP£/kg	GenBank			Mislabelling?
					Species identified	Similarity (%)	Sequence length (bp)	
88- SB	Sea bass	Fresh whole	SU	11-12	*Dicentrarchus labrax*	98	383	No
89- SB	Sea bass	Fresh whole	SU	11-12	*Dicentrarchus labrax*	99	392	No
90- SB	Sea bass	Fresh whole	SU	11-12	*Dicentrarchus labrax*	99	389	No
91- SB	Sea bass	Fresh whole	SU	11-12	*Dicentrarchus labrax*	99	386	No
92- SA	Salamon	Filleted frozen	SU	15-16	*Salmo salar*	100	397	No
93- SA	Salmon	Filleted frozen	SU	15-16	*Salmo salar*	100	394	No
94- SA	Salmon	Filleted frozen	SU	15-16	*Salmo salar*	99	396	No
95- SA	Salmon	Filleted frozen	SU	15-16	*Salmo salar*	100	387	No
96- SA	Salmon	Filleted fresh	SU	16-18	*Salmo salar*	99	395	No
97- SA	Salmon	Filleted fresh	SU	16-18	*Salmo salar*	100	389	No
98- SA	Salmon	Filleted fresh	SU	16-18	*Salmo salar*	100	381	No
99- SA	Salmon	Filleted fresh	SU	16-18	*Salmo salar*	99	387	No
100- SR	Sardine	Fresh whole	SU	10-11	*Sardinella aurita*	99	368	No
101- SR	Sardine	Fresh whole	SU	10-11	*Sardinella aurita*	99	374	No
102- SR	Sardine	Fresh whole	SU	10-11	*Dicentrarchus labrax*	86	378	UM
103- SR	Sardine	Fresh whole	SU	10-11	*Sardinella aurita*	99	396	No
104- SI	Sultan Ibrahim	Fresh whole	SU	12-14	*Nemipterus bathybius*	85	389	UM
105- SI	Sultan Ibrahim	Fresh whole	SU	12-14	*Cyprinus carpio*	88	372	UM
106- SI	Sultan Ibrahim	Fresh whole	SU	12-14	*Nemipterus bathybius*	87	389	UM
107- SI	Sultan Ibrahim	Fresh whole	SU	12-14	*Carassius auratus*	97	380	UM
108- KF	Kingfish	Filleted fresh	SU	16-18	*Scomberomorus commerson*	96	375	UM
109- KF	Kingfish	Filleted fresh	SU	16-18	*Scomberomorus commerson*	94	396	UM
110- KF	Kingfish	Filleted fresh	SU	16-18	*Scomberomorus commerson*	95	376	UM
111- MA	Mackerel	Fresh whole	SU	12-14	*Rastrelliger kanagurta*	99	389	No
112- MA	Mackerel	Fresh whole	SU	12-14	*Rastrelliger kanagurta*	99	369	No
113- MA	Mackerel	Fresh whole	SU	12-14	*Rastrelliger kanagurta*	98	388	No
114- NP	Nile perch	Filleted fresh	SU	14-16	Failed to amplify	---	---	---
115- NP	Nile perch	Filleted fresh	SU	14-16	*Cyprinus carpio*	90	386	UM
116- NP	Nile perch	Filleted fresh	SU	14-16	*Cyprinus carpio*	97	385	UM
117- CF	Cuttlefish	Filleted fresh	SU	12-14	*Labiobarbus lineatus*	83	375	UM
118- CF	Cuttlefish	Filleted fresh	SU	12-14	*Cyprinus carpio*	97	368	UM
119- CF	Cuttlefish	Filleted fresh	SU	12-14	*Sardinella lemuru*	87	367	UM
120- CF	Cuttlefish	Filleted fresh	SU	12-14	*Garra lamta*	85	363	UM

[1]Samples 1-87 were sold as local carp fish species, while samples 88-120 were sold as imported fish species. FCC; farmed common carp, WCC, wild common carp, WUN, wild unknown species, SH, shabbout, SB, Sea bass, SA, Salmon, SR, Sardine, SI, Sultan Ibrahim, KF, Kingfish, MA, Makerel, NP, Nile perch, and CF, Cuttlefish. [2]DF, direct from farm, FM, fishmongers, RE, restaurant, SM, street markets, SU, supermarkets, UM, unreliable match

Table 4.5: List of samples sequenced at the COI region with their matched identification results from Genbank and BOLD (n=23)

Sample code[1]	Fish sold as (local name)	GenBank		BOLD		Sequence length (bp)	Mislabelling?
		Species identified	Similarity (%)	Species identified	Similarity (%)		
5- FCC	Common carp	*Cyprinus carpio*	99	*Cyprinus carpio*	100	620	No
28- FCC	Common Carp	*Cyprinus carpio*	99	*Cyprinus carpio*	100	647	No
35- FCC	Common Carp	*Cyprinus carpio*	99	*Cyprinus carpio*	100	608	No
37- WCC	Common Carp	*Cyprinus carpio*	99	*Cyprinus carpio*	100	643	No
58- WCC	Common Carp	*Cyprinus carpio*	99	*Cyprinus carpio*	99.4	605	No
79- SH	Shabbout	Arabibarbus grypus	99	Sequence not available	-----	640	No
84-SH	Shabbout	Arabibarbus grypus	99	Sequence not available	-----	617	No
87-SH	Shabbout	Arabibarbus grypus	99	Sequence not available	-----	613	No
102-SR	Sardine	Sardinella longiceps	99	Sardinella longiceps	100	620	No
104- SI	Sultan Ibrahim	*Nemipterus japonicas*	99	*Nemipterus japonicus*	99.47	640	No
105- SI	Sultan Ibrahim	*Nemipterus peroneii*	98	*Nemipterus peronii*	100	651	No
106-SI	Sultan Ibrahim	*Nemipterus Japonicas*	99	*Nemipterus japonicus*	99.84	664	No
107- SI	Sultan Ibrahim	*Nemipterus Japonicas*	99	*Nemipterus japonicus*	99.83	619	No
108- KF	Kingfish	*Scomberomorus Commerson*	100	*Scomberomorus commerson*	100	617	No
109- KF	Kingfish	*Scomberomorus Commerson*	99	*Scomberomorus commerson*	99.83	637	No
110- KF	Kingfish	*Scomberomorus Commerson*	99	*Scomberomorus commerson*	100	651	No
114- NP	Nile perch	*Lates niloticus*	100	*Lates niloticus*	100	649	No
115- NP	Nile perch	*Lates niloticus*	100	*Lates niloticus*	100	645	No
116- NP	Nile perch	*Lates niloticus*	99	*Lates niloticus*	100	657	No
117-CF	Cuttlefish	*Sepia pharaonic*	99	*Sepia pharaonis*	99.81	607	No
118- CF	Cuttlefish	*Sepia pharaonic*	99	*Sepia pharaonic*	99.81	613	No
119- CF	Cuttlefish	*Sepia pharaonic*	99	*Sepia pharaonic*	99	604	No
120- CF	Cuttlefish	*Sepia pharaonic*	99	*Sepia pharaonic*	99.06	606	No

[1]FCC, farmed common carp, WCC, wild common carp, SH, shabbout, SR, Sardine, SI, Sultan Ibrahim, KF=Kingfish, NP, Nile perch, CF, Cuttlefish

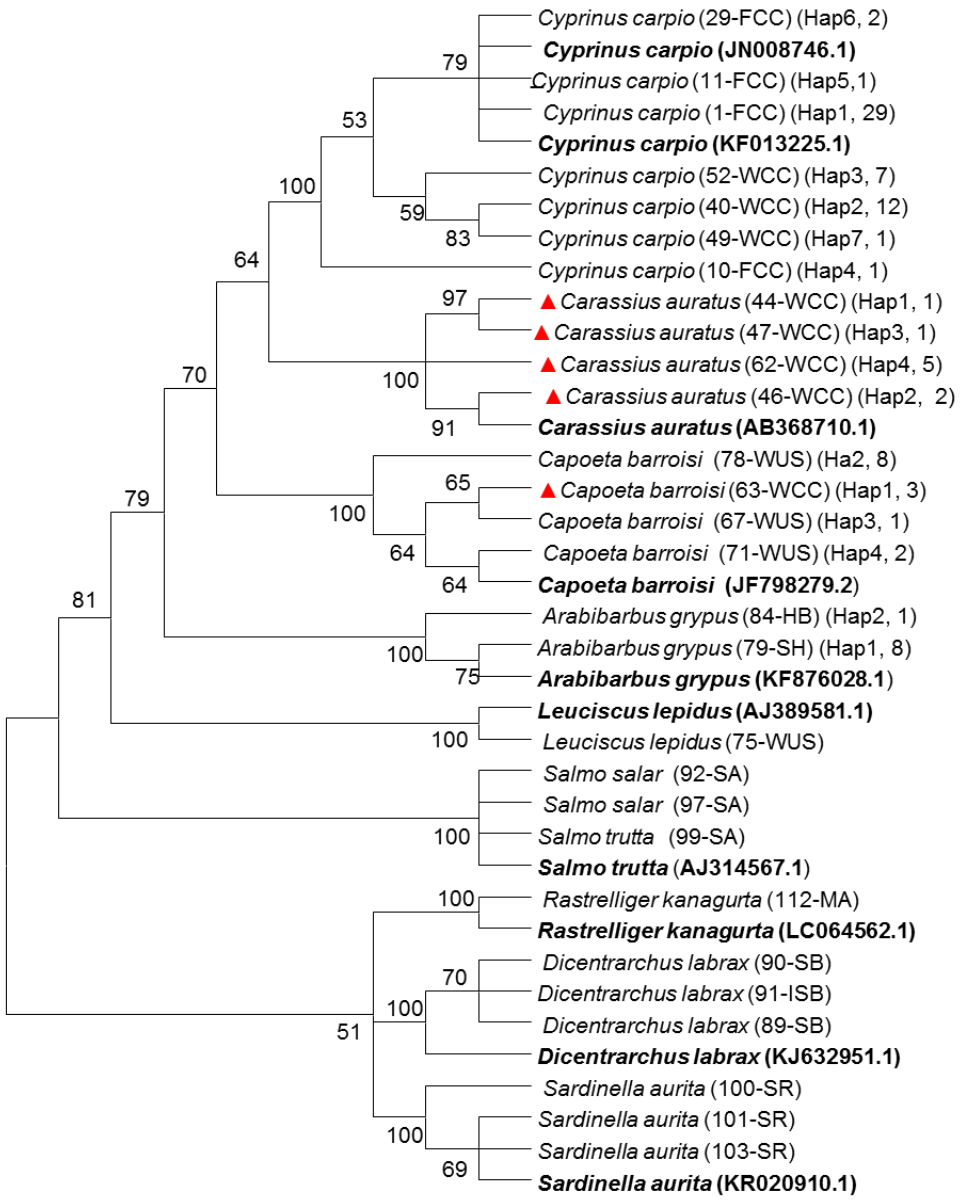


Figure 4.3: Unrooted Neighbour-Joining tree (333bp in the final dataset), showing the phylogenetic relationships between genetically identified samples (n=28) at the Cytb region and validated reference sequences retrieved from GenBank with accession numbers (n=10). Samples are labelled with the sample code and the declared name (see Table 1 for details) (first brackets), while the second code refers to the haplotype and numbers of samples of that haplotype (for species with >4 samples). Samples with red symbol (▲) were detected to be mislabelled. Scale bar used was 0.02. The scale bar corresponds to the number of base substitutions or residue per site

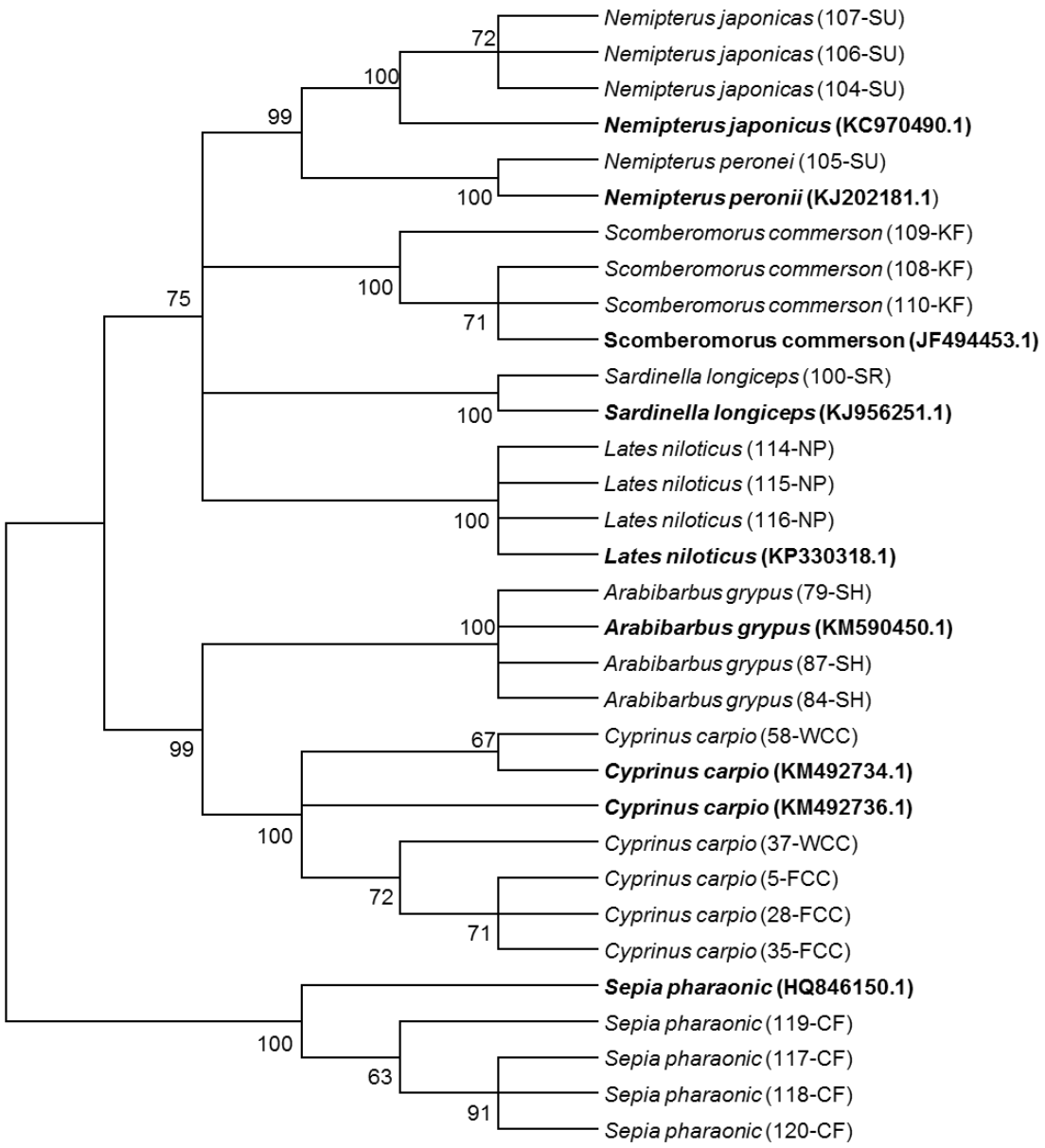


Figure 4.4: Unrooted Neighbour-Joining tree (559bp in the final dataset), showing the phylogenetic associations between genetically identified samples (n=23) at the COI region and validated reference sequences retrieved from GenBank with accession numbers (n=9). Samples are labelled with the sample code and the declared name (see Table 1 for details). Scale bar used was 0.02. The scale bar corresponds to the number of base substitutions or residue per site

4.4 Conclusion

This work represents the first DNA barcoding analysis to assess the potential mislabelling of fish species sold in KRI markets. Our study confirmed that in most cases DNA barcoding using the Cytb and/or COI regions is reliable and that it can provide fast, efficient and unambiguous identification for samples comprising a wide range of fish species and processed formats. The cases of mislabelling identified were probably unintentional and due to morphological similarities, but these results confirm that fish mislabelling and/or substitution is a generalized practice across the world and adds the KRI to the long list of countries where it happens, and provides further evidence that regulations on food fraud practices need to be strengthened and new policies and guidelines implemented to increase traceability of fish products.

Most of the analysed samples were grouped with others from the same species in phylogenetic trees, implying that the sequence data and identifications made in this study are indeed correct and robust. However, wild and farmed common carp origin could not be discriminated on the basis of their position in the phylogenetic trees, and the question remains to whether farmed fish is being sold as wild fish for economic gain.

Given the lack of resources available for full traceability and enforcement, implementation of a reliable DNA technique for fish authentication may discourage deliberate replacement in seafood markets in Kurdistan, which in turn may lead to reductions in seafood mislabelling and protect consumers from fraud. Furthermore, additional compliance with legislation requirements in KRI is timely to ensure the acceptability of the fish authentication.

Chapter 5

Applications of fingerprinting compositional profile and near-infrared spectroscopy (NIRS) for the authentication origin of wild and farm common carp (*Cyprinus carpio)*

5.1 Introduction

Fish and fish products play a useful role in a healthy and balanced diet. Health benefits are related to its nutritional richness, including protein and long-chain omega-3, PUFAs, as well as micronutrients, including vitamins, and minerals (Weichselbaum *et al.*, 2013). A recommendation from the department of health in the UK is to eat at least two portions of fish (140 g each) per week, one of which should be oily fish (SACN, 2004).

Common carp (*Cyprinus carpio*) has been one of the most cultivated fish species all over the world (Komen, 1990, Guler *et al.*, 2008, Chen *et al.*, 2009) and accounts for up to 10% (over 3 million metric tons) of global annual freshwater aquaculture production (FAO, 2012, Xu *et al.*, 2014). It is among the main cultivated fish species in Asian and European aquaculture (Zhou *et al.*, 2003), and the main cultured fish species in Iraq (Kitto and Tabish, 2004). The popularity may be linked to their fast growth rate, easy farming (Yeganeh *et al.*, 2012), and high feed efficiency ratio (Afkhami *et al.*, 2011). Farmed fish have different rearing systems, stocking densities and feeding systems that are different to those of wild fish (Arechavala‐Lopez *et al.*, 2013).

Carp, and particularly common carp, is one of the most important freshwater fish species in Iraq (Nasir, 2013). Aquaculture activity in the Kurdistan region of Iraq started in the 1960s with the common carp at the Dokan and Darbandikhan dams. However, the economic success was only recognized in 1998 when the FAO established two stations for fish production in the region (Omar, 2011). The increased production of common carp has raised concerns over the quality of farmed fish, in comparison with wild fish (Yeganeh *et al.*, 2012).

Some reports showed that the majority of wild fish species have premium prices over farmed fish (Bell *et al.*, 2007, Morrison *et al.*, 2007, Arechavala‐Lopez *et al.*, 2013). Farmed and wild fish may carry different hazards and are therefore subjected to different regulations and analytical controls. For instance, farmed fish may contain residues of veterinary drugs which are unlikely to be present in wild fish, while wild fish may contain parasites that are harmful to humans, but rarely present in farmed fish (Martinez *et al.*, 2010).

In addition, the presence of toxic contaminants is lower in farmed fish such as salmon and can be more easily controlled and monitored in farmed fish than in wild fish (Cahu *et al.*, 2004). Fish muscle often serves as a good source of essential minerals (Lal, 1995). Furthermore, due to their importance in human diets, fish must be carefully screened to ensure that dangerous levels of heavy metals are not being displaced into the human diet (Rahman *et al.*, 2012), and the assessment of fish origin is a security measure to protect consumers and avoid fraud practice in seafood industry (Ottavian *et al.*, 2012).

Although it is widely believed that wild fish acceptability is better than farmed fish, generally consumers expect farmed fish to be equivalent or superior to the wild fish (Yeganeh *et al.*, 2012). However, it has not been confirmed whether farmed fish is safer than wild fish or vice versa (Verbeke and Brunso, 2005). There are some reports on consumers' perceptions and beliefs regarding the differences between wild and farmed fish of different species and in different countries using quantitative and/or qualitative tools (Verbeke and Brunso, 2005, Verbeke *et al.*, 2007, Schlag and Ystgaard, 2013, Vanhonacker *et al.*, 2013, Claret *et al.*, 2014). Raising concerns among fish consumers is whether the

nutritional value of farmed and wild fish is equivalent, particularly the lipid composition (Cahu *et al.*, 2004).

To tighten the traceability and authenticity in the production chain, the EU directive (EC/2065/2001) requires mandatory information for a full characterization of the marketing fish with clear labelling information including production method of fish (i.e. wild/farmed), geographical origin and fish species. It is aimed at avoiding mislabelling or substitution wild fish with farmed fish and protecting consumer's confidence and health (European Commission, 2001, Delgado *et al.*, 2003). Strict labelling regulations are required by several importing countries in order to assurance the safety, traceability and authenticity of fish products (Mohanty *et al.*, 2013).

Therefore, the implementation of analytical methods to discriminate between wild and farmed fish may help to ensure correct labelling information and avoid fraud (Martinez *et al.*, 2010). Several analytical techniques have been successfully applied including: morphological examination (Solem *et al.*, 2006), genetic analysis (Alarcón *et al.*, 2004, Karaiskou *et al.*, 2009, Hosseinnia *et al.*, 2014), NMR (Mannina *et al.*, 2008), isotopic analysis and trace element profile (Bell *et al.*, 2007), analysis of chemical composition (Chen *et al.*, 1995, Martinez *et al.*, 2007, Li *et al.*, 2011) and volatile compounds using gas chromatography (Mahboob *et al.*, 2009).

In the quality measurement system, NIR spectroscopy methods have plenty of applications in the field of agriculture and the food industry, including food authentication or classification (Downey, 1996, Ballabio and Todeschini, 2009).

In the last decades, the attention has focused on development of NIRS for both quantitative analyses and chemical composition linked to qualitative analyses (discriminant analysis), and has been widely used to discriminate between wild and farmed fish (Xiccato *et al.*, 2004, Ottavian *et al.*, 2012), rearing systems (Xiccato *et al.*, 2004), and for differentiating between fresh and frozen-thawed fish (Uddin and Okazaki, 2004, Fasolato *et al.*, 2012). It has been used to predict chemical composition in fish (Cozzolino *et al.*, 2002b) and to determine macro and micro elements in a wide range of agriculture products and foods (McClure, 2003).

The spectrum of organic materials gives a global signature of composition based on the assessment of the organic chemical structures containing O-H, N-H and C-H bonds (Murray and Cowe, 2004). This NIR spectrum has its origin in the different vibration modes of the molecules which are caused by their interaction with electromagnetic radiation absorbed at wavelength between 750 and 2500 nm. The use of chemomettrics allows the relevant information contained in the NIR spectra to be extracted and used in the development of calibration models that permit the prediction of the components' composition (Collell *et al.*, 2010).

Not many reports illustrate the use of NIR spectroscopy technique to predict the proximate and mineral compositions and to discriminate between wild and farmed origins of common carp. The overall aim of the present study was to evaluate the potential of NIR spectroscopy as an alternative technique to quantitatively predict the proximate and mineral composition in muscle fish as well as discriminate between wild and farmed common carp.

The specific objectives were to:

1- To quantify the proximate and mineral composition of muscle fish using NIRS calibration model.

2- To develop robust NIRS calibration models based on the proximate and mineral composition of muscle fish and their NIR spectra to investigate their potential origin discrimination of wild and farmed carp.

3- To measure toxic mineral levels in the fish muscle to assess their risk of accumulation in the fish and potential entry into humans.

5.2 Materials and method

5.2.1 Sample collection and preparation

A total of 56 whole fresh samples (n=56) declared as wild (n=29) or farmed (n=27) common carp were purchased from different markets in Erbil province/ KRI between December 2014 and January 2015. These samples were listed on Table 4.4 for further details and labelled as farmed common carp (1-FCC to 27-FCC), and wild common carp (36-WCC to 66-WCC).

Samples were purchased at fishmongers, street shops, and direct from farmers after reaching a weight greater than 600g (i.e., their commercial size). The specimens were transported to the laboratory in an ice box, below 4°C and within 5h. After skinning, filleting, and washing, about 250-300g of white muscle portion (dorsal muscle) was cut and added to a labelled container and kept cold (4°C) until further analysis. All the samples were treated under the same conditions.

Initially all samples were oven-dried in an electric oven between 68-72°C overnight. Dried samples were packed in labelled flexible plastic films, sealed to protect from air oxidation, covered with a thin layer of aluminium foil to protect from light oxidation, and stored in the freezer prior to shipping to the UK. After an approximate of 24h of shipping to the UK, they were freeze dried for about 48h at -40°C and ground to obtain a homogeneous sample size. The samples were stored in a sealed plastic bag prior to analysis at room temperature.

5.2.2 Sample selection, specification and identification

During the collection of fish samples at selling points (fishmongers and street markets), several observations were made that fish species were not labelled

properly regarding species identity and origin of the production method. The declaration of fish origin (wild or farmed) was ambiguous, and carp samples were often sold without a species name. However, samples were purchased based on trust and the price differences between wild and farmed as the wild had premium prices over farmed fish (Table 4.4). This ambiguity is potentially concerning for consumers at commercial markets in KRI.

However, all purchased samples (n=56) were subjected to genetic analysis using DNA barcoding (described in Chapter 4, Table 4.4) as a method of species identification. From 56 common carp samples used in this study, only ten samples were mislabelled (three samples were identified as *Carassius auratus* and seven as *Capoeta barroisi*), while the rest (*46*) were identified as they were sold, as common carp (*Cyprinus carpio)* (Figure 5.1).

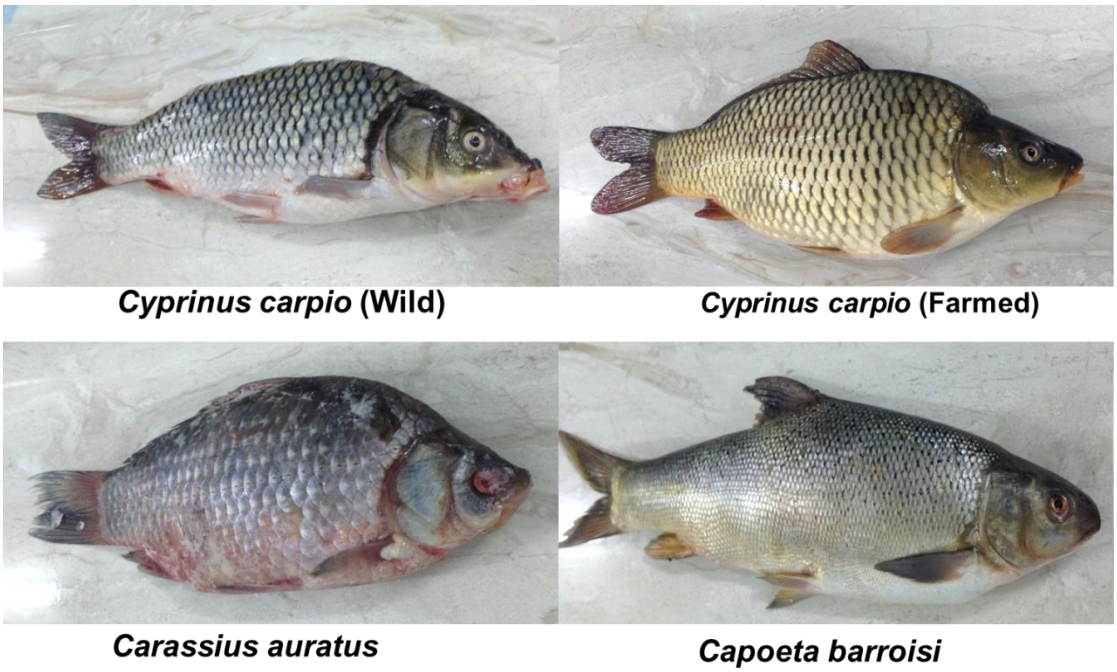


Figure 5.1: Pictures of collected fish species

5.2.3 Determination of chemical composition of fish muscle

Twenty two samples out of 56 were determined in triplicate for their chemical composition (moisture, protein, lipid and ash) using standard methods (AOAC, 2003) (described in Chapter 3, section 3.2.4). These 22 samples were used for the NIRS calibration model set up and validation.

5.2.4 Analysis of mineral composition using ICP-OES and ICP-MS

5.2.4.1 Samples digestion

The mineral contents (K, P, S, Na, Ca, Mg, Fe, Zn, Cr, Co, Cd, Cu, Se, As, Pb, Mn and Hg) of dried muscle fish were determined according to the method described by Nielsen (1998). Kjeldahl digestion tube was first washed, rinsed and soaked in 2% HNO_3 solution and left overnight. After that, it was rinsed with distilled water and oven dried before using. Approximately 0.25 g of freeze dried samples were weighed precisely and directly placed into a cleaned micro Kjeldahl boiling tube and digested with 10 ml of HNO_3 (70%). Digestion was conducted in Gerhardt Kjeldathem 40 tubes digestion block (Gerhardt Laboratory Instruments, Bonn, Germany) at 60°C for one hour; the temperature was raised to 90°C for another hour, and then raised to 110°C for 30 min, and finally to 135-140°C for up to 4h.

The digestion process was performed in the scrubber unit Gerhardt Turbosog unit in which acid fumes was neutralised through a 15% NaOH. Digestion was confirmed visually by the presence of a clear pale green solution. After digestion the tubes were removed from the hot-block and allowed to cool down at room temperature. After cooling, sample solutions were quantitatively transferred into

a 50 ml polypropylene vial after diluted to exactly a volume of 50 ml with deionized water. Triplicates for of each sample were carried through the digestion reaction and two samples of blanks were analysed using the same procedure to check the purity of reagents and any possible contamination.

5.2.4.2 Determination of minerals content

After digestion, five standard solutions were prepared at levels 0, 0.1, 4, 10, 40 mg/l for macro-minerals (K, P, S, Na, Ca, Mg, Fe, Zn) using iCAP 7400 series, inductively coupled plasma optical emission spectrophotometry (ICP-OES). The second standard solution was prepared at level 0, 10, 20, 40, 100 µg/l for micro-minerals (Mn, Cu, Se, As, Pb, Cr, Cd, Co and Hg) using Thermo Scientific X series 2, (ICP-MS, Hemel Hempstead, UK). For each sample a triplicate was taken, and the two sample blanks were also analysed (a procedural blank was prepared in the same way of the standard solution, but omitting the sample) in each run with both instruments.

In order to validate our methods, the two (TORT-2 and DOLT-4) certified reference materials (National Research Council of Canada, Ottawa, Canada) were analysed for micro-minerals analysed with ICP-MS. However, due to the unavailability of the certified values of some macro-minerals, they were not analysed for the validation of ICP- OES method.

The operating conditions employed for both instruments are described in Table 5.1. The mineral concentrations were reported as mg/g (macro-element) and µg/g (micro-element) on dry weight bases that could provide more stable basis for comparison than wet weight.

However, for the comparison of mineral concentration with the international standards, where the concentrations are mostly given on wet weight basis, an equation was used in order to convert the dry weight into wet weight based on the average of moisture content measured in muscle fish (wild common carp= 79.7±2.0, farmed common carp= 77.2±3.2, *Capoeta barroisi* =78.7±1.0 and *Carassius auratus* =76.4±1.9) according to the following formula:

$$\text{Wet weight concentration} = \text{dry weight concentration} \times \frac{100 - \text{Moisture content (\%)}}{100}$$

Table 5.1: Operation parameters used for the determination of all minerals by ICP-MS and ICP-OES

Equipment	**Parameters**		**Values**
ICP-MS (X Series 2, Thermo Fisher Scientific)	Peristaltic pump speed/ mL min^{-1}		1.2
	Nebulizer type		Burgener
	Spray chamber		Conical
	Radio frequency power		1350
	Forward power/w		1400
	Dwell time (ms)		10
	Collision cell gas flow (mL min-1) 7 % H2 in He		3.6
	Gas flow (L. min^{-1})	Coolant	13
		Auxiliary	0.75
		Nebulizer	0.84
ICP-OES (iCAP 7400 series, Thermo Fisher Scientific)	Peristaltic pump speed ml /min		1.1
	Nebulizer type		Burgener (MiraMist)
	Spray chamber		Cyclone
	Exposure time		2
	Radio frequency power (W)		1150
	Viewing high (mm)		12
	Wavelength (nm)		177.4
	Gas flows/L min^{-1}	Coolant	12
		Auxiliary	0.5
		Nebulizer	0.5

Table 5.2: Summary of certified values for reference materials TORT-2 and DOLT-4 and mean values experimentally obtained by ICP-MS (All values are mg/kg dry wt., n=6)

Elements	TORT-2			DOLT-4		
	Certified value	Found value	Recovery (%)	Certified value	Found value	Recovery (%)
Cu	106±10.0	92.81±4.47	88	31.2±1.1	28.87±1.30	93
Se	5.63±0.67	5.14±0.20	91	8.3±1.3	7.32±0.14	88
Mn	13.6±1.20	13.17±0.91	97	---	---	---
Cr	0.77±0.15	0.74±0.08	96	1.4	1.38±0.38	99
As	21.6±1.80	19.82±1.10	92	9.66±0.62	8.76±0.33	91
Pb	0.35±0.13	0.34±0.02	97	0.16±0.04	0.17±0.03	106
Co	0.51±0.09	0.51±0.03	100	0.25	0.24±0.02	96
Cd	26.7±0.6	24.94±1.15	93	24.3±0.8	21.47±0.82	88

5.2.5 NIR analysis

5.2.5.1 Obtaining NIR spectra

About 20g of homogeneous freeze dried sample was placed in a 50 mm diameter ring cup holder and scanned in reflectance mode at 2 nm intervals over the NIR spectral wavelength ranged from 12500- 4000 cm^{1-} (corresponding to a wavelength interval of 800-2500 nm) (Figure 5.5) using a scanning monochromator instrument- Matrix-I spectroscopy (Bruker Optics

Gmbh, Germany, 2006). Bruker OPUS Software (Version 6) was used for all data collection. The NIR spectra of the 56 samples (each in triplicate) of muscle fish of wild and farmed of common carp are shown in Figure 5.5, and the average spectra of wild and farmed common carp are shown in Figure 5.6. The spectrum of each sample represented the average of 64 multiple scans, which could reduce sampling error. Spectral data were stored as the logarithm of the reciprocal of reflectance [log (1/R)]. The time necessary for a NIR measurement was about 15s.

5.2.5.2 Development of calibration model based on PLS

Bruker OPUS software (version 6) was performed to create the calibration model using spectral data extracted from NIR and the reference data of known concentrations measured in the lab, in order to quantitatively predict the unknown concentrations of chemical composition in fish samples. The same wavelengths used for measuring NIR spectra were selected.

The PLS regression method was used to develop the calibration model in the present study (Abdi, 2003) as the most commonly used regression algorithm in the field of chemometrics in spectroscopy technique (Burns and Ciurczak, 2007). Detailed information on the mathematical background and principle of PLS regression has been described elsewhere (Martens and Jensen, 1982, Nørgaard *et al.*, 2000, Abdi, 2003). The main advantage of using PLS over PCA is to reduce the complexity of the model by using fewer principal components that contain more related information (Martens and Jensen, 1982). Furthermore, PLS also takes into account the correlation between the spectra data and the

component concentration, while extracting the latent variables from the original data matrix, thus latent variables directly refer to the given component (Balabin *et al.*, 2007).

For building the predictive model properly, a sufficient amount of information must be provided, in terms of NIR spectra of the calibration. Two separate models were developed using reference data for each of minerals (n=33) and chemical composition (n=22) that were selected to be representative for the system. As a rule there is no general recommendation that can be followed concerning the rule of thumb to determine the optimum number of selected samples in calibration set. The selected number depends on the complexity of the correction (wavelength shift, intensity offset) and on the algorithms used (Naes *et al.*, 1986).

However, according to the Bruker Optik (2006) software, for one component system a minimum of 20 samples should be measured, and multicomponent systems require a large number of calibration samples. The reason for developing two separate models was due to the NIR requirement that the only prerequisite of NIRS application for quantitative purposes is to develop a separate reliable calibration model for every commodity, constituent or quality measurement (Williams, 2001). The basic steps for creating the predictive models and validating were summarised (Figure 5.2).

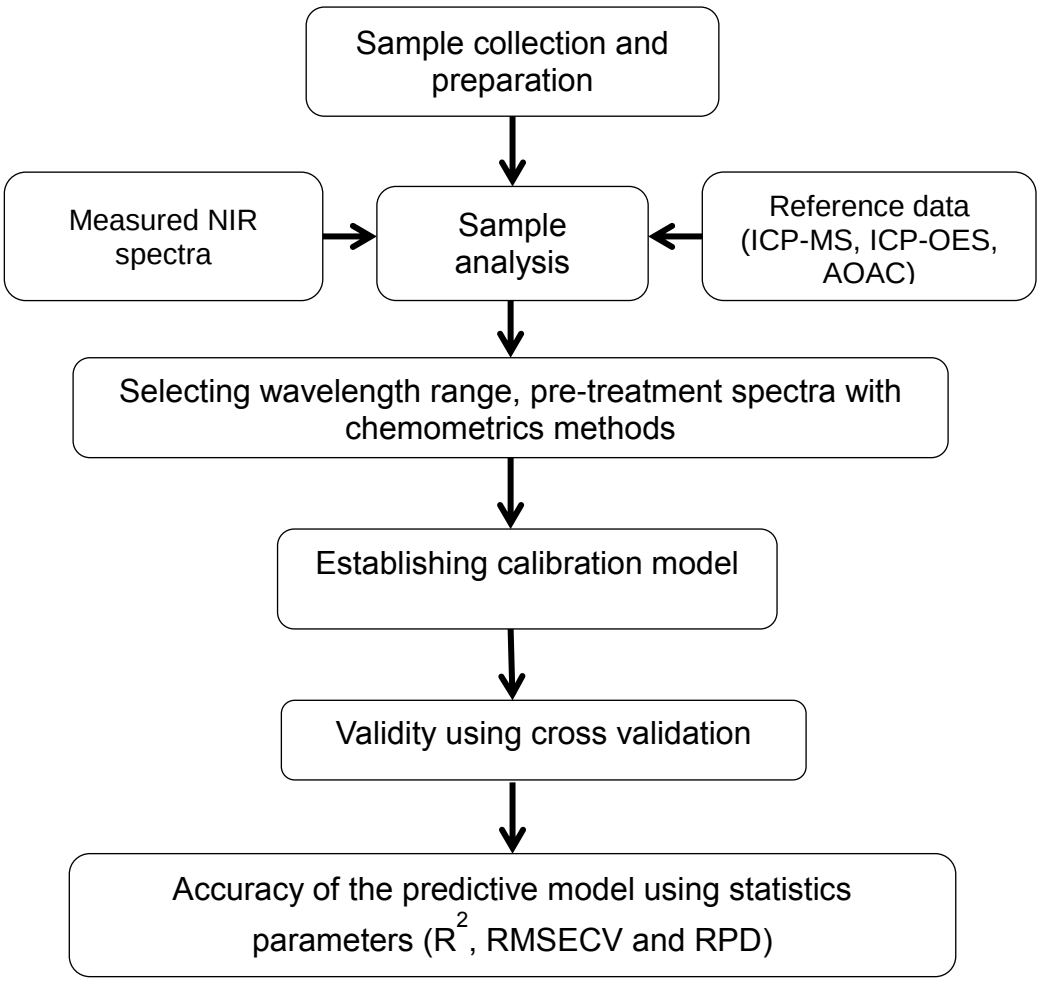


Figure 5.2: Diagram of the methodology followed to create NIR predictive model

5.2.5.3 Validation of the calibration model

The validation process was performed to calculate the difference between NIR spectroscopy prediction results obtained for the constituents, properties and the measurements obtained for the reference method or known identities (Næs and Isaksson, 1991). The rank number used during the validation indicates the number of factors which are used by the model, and the root mean square error of cross validation (RMSECV) is dependent on the rank of the model (Figure 5.4). The calibration model was internally validated using the same sample set

as used for calibration development (Martens and Naes, 1992). The model was validated to test its reliability of prediction using the full cross-validation (leave-one-out) in order to avoid over-nor under- fitting of the model.

In this method, one spectrum was omitted from the dataset, and the remaining spectra were used to build the model, which was then applied to the omitted "validation" spectrum. The excluded sample (spectrum) was then reintroduced, and another spectrum was removed and the calibration and prediction process was repeated. This process of omitting one spectrum in turn was repeated until every sample had been used for both calibration development and validation which finally produced a complete series of predictions for the entire dataset.

5.2.5.4 Spectra pre-processing

To optimize the calibration accuracy and ensure good correlation between the spectral data and concentration value, as well as to remove any irrelevant information that could not be handled properly by the regression techniques, data pre-treatment was performed in OPUS Software (Version 6) that would better adhere to Beer's law, which states that absorbance and concentration are linearly correlated (Rinnan *et al.*, 2009).

Because there is no general recommendation as to which method is suited best for a pre-processed data set (Bruker Optik, 2006), spectra were subjected to different pre-processing methods including vector normalization (VN), multiplicative scatter correction (MSC) (Dhanoa *et al.*, 1994), first and second derivative (Shenk *et al.*, 1992), minimum and maximum normalization, straight line subtraction, constant offset elimination, or a combination of these options.

However, the better results were found empirically by applying the VN and MSC methods which are used to achieve a better match of single spectra to the mean spectrum of the set (Bruker Optik, 2006).

5.2.5.5 Evaluation of the predictive model

The statistics most often used for quantitative NIR analysis are include the RMSECV, the coefficient of determination (R^2), and the ratio of standard deviation to standard error of prediction (RPD) (Osborne *et al.*, 1993, Williams, 2001). These statistics are used to evaluate the overall accuracy of the prediction model as follows;

1- The residual errors between the predicted and the actual concentration of samples were calculated in both calibration and cross-validation conditions, and the predictability of the best PLS regression model was identified at the minimum values of the RMSECV.

$$RMSECV = \sqrt{\frac{\Sigma[y\,(pred) - y\,(act)]}{N}}$$

Where N is the number of spectra (sample) in the calibration set, and the y^{pred} is the predicted value by cross validation and y^{act} is the actual value.

2- Coefficient determination of cross validation (R^2), which indicates the closeness of fit between the NIR and reference data over the range of composition, which should be close to 1 (Williams, 2001), as described in Appendix 5.1. The R^2 should be high (0.90) to indicate a good prediction

capability, while with a low value (0.64) it is not possible to obtain consistently high accuracy by NIR spectroscopy analysis (Manley *et al.*, 2008).

3- The residual predictive deviation (RPD) statistic is the ratio of standard deviation (SD) of the reference values to SEP of the validation RPD= SD/SEP. The smaller the error of prediction is, compared to the variance of the reference values, the larger the RPD value is and therefore, the better the performance of the model (Appendix 5.2). The model is considered good for screening with a RPD > 3, good for quality control with a RPD > 5, and excellent for all analytical tasks with a RPD > 8 as described in Table 5.5 (Williams, 2001, Conzen, 2006).

5.2.6 Statistical analysis

Bruker OPUS^{TM}- QUANT software (version 6) was used for all data collection and analysis (measuring NIR spectra, selection of wavelengths, mathematical pre-treatments, PLS regression, and full cross validation (leave-one-out). Correlations among data obtained for coefficient of determination in calibration (R^2) were calculated by plotting scatter using Excel. The results of the standard methods and analysed NIR spectroscopy data were statistically analysed using paired t-test in Minitab v.17 (Minitab Ltd., Coventry). The results are presented as mean ± SD. The results were submitted to analysis of variance (One- way ANOVA), at 0.05 significant level, and the mean values were compared by Tukey's test.

PCA is one of the most popular multivariate statistical techniques, often used to characterise foodstuffs according to their origin (Monfreda, 2012). PCA was performed (Minitab v. 17) to understand the differences of the proximate and minerals composition according to the source of origin (wild and farmed) and

species identity. PCA was used to extract the most relevant information from the data and to represent it as a set of new orthogonal variables, called principal components, that display the pattern of similarity of the observations and of the variables as points in maps (Jackson, 1991, Abdi and Williams, 2010). PCA allows an easy visualization of all the information contained in a data set and to find out in what respect one sample is different from another and which variable contribute most to this difference (Beebe *et al.*, 1998).

5.3 Results and discussion

5.3.1 Accuracy of the NIRS predictive model

Results of the prediction model are shown in Table 5.3. In general, all four components of proximate composition had a very good correlation, and were comparable to the values (RPD and R^2) for application of NIR (Williams, 2001). Generally a R^2 value larger than 0.91 indicates a good correlation (Williams, 2001, Elfadl *et al.*, 2012), which is obtained for all proximate chemical components in the present study.

The RPD value is a measurement of the ability of an NIRS model to predict a constituent efficiently (Abdi and Williams, 2010). An RPD value > 3, as observed for most of the analysed components, indicated the possibility of obtaining an accurate quantitative estimation which can be used for screening of food product for quality control. Because the values for protein and fat were even higher (>8), models were considered to excellent for predictions and could be used for these applications (Table 5.3 and Figure 5.3, 5.4) (Williams, 2001, Conzen, 2006). The correlations for moisture and ash were also good

with R^2 values of 0.98 and 0.96, and RPD values 6.36 and 4.97 respectively, indicating that the accuracy of the calibration model would be good for processing control (moisture) and adequate for screening purposes (ash).

This result indicates that NIRS could be used as potential alternative technique to predict or quantify the proximate chemical composition in fish samples accurately with the obtained parameters.

The best correlations within macro-minerals were observed with Mg, S, P and K, while the values of R^2 and RPD for each Na, Ca, Zn and Fe were considered insufficient for most applications and are not recommended to be used for NIR calibration (Table 5.3) and (Figure 5.4). For Na, the R^2 0.88, however, even the value higher than 0.83 can show that the robustness of the prediction of calibration model is still maintained (Elfadl *et al.*, 2012). Generally, if the mineral measured is bonded to organic compounds, the distortion of the spectrum may be detectable by certain wavelengths, suggesting that NIR spectroscopy might predict some elements (Cozzolino *et al.*, 2011).

This study has shown that NIRS spectra can be used to predict the concentration of some macro-minerals (K, P, S, Mg and Na) in muscle fish (Figure 5.3). However, the NIRS calibration model developed for Ca, Zn, Fe and for all analysed micro-minerals (not shown) were not suitable for quantitative routine testing, and eventually could not be used for discrimination between wild and farmed common carp. Therefore, further development with larger data sets, or a narrower source of samples, may be required for the calibration to become more stable and sufficient to predict the components (Table 5.3).

Table 5.3: Chemical comparison (%) using NIR data and standard methods (n=22 for proximate chemical composition) and (n=33 for minerals composition)

Components (%)	Data		Paired-t-test (P=0.05)					Parameters for validating the model		
	x-Std	x-NIR	Deviation (Mean)	SD	P-value	SE mean	T-value	R^2	RPD	RMSECV
Moisture	4.13	4.08	0.05	0.35	0.28	0.04	1.09	0.98	6.36	0.352
Protein	69.61	69.78	-0.17	1.17	0.24	0.14	-1.19	0.99	13.1	0.86
Fat	21.56	21.58	-0.02	1.14	0.85	0.14	-0.19	0.99	13.7	0.97
Ash	4.39	4.51	-0.12	0.72	0.18	0.08	-1.36	0.96	4.97	0.76
K	13.34	13.32	0.015	1.06	0.89	0.11	0.14	0.91	3.24	1.06
P	7.52	7.51	0.006	0.53	0.89	0.05	0.13	0.93	3.66	0.53
S	7.27	7.27	-0.007	0.31	0.81	0.03	-0.24	0.94	4.07	0.31
Mg	1.05	1.05	0.001	0.06	0.87	0.01	0.16	0.95	4.3	0.06
Na	2.05	2.05	0.002	0.27	0.94	0.03	0.07	0.88	2.86	0.27
Ca	1.79	1.79	0.005	0.57	0.94	0.06	0.08	0.43	1.33	0.57
Zn	0.034	0.034	0.000	0.008	0.80	0.001	0.25	0.57	1.53	0.01
Fe	0.033	0.033	0.000	0.006	0.98	0.001	0.03	0.51	1.43	0.01

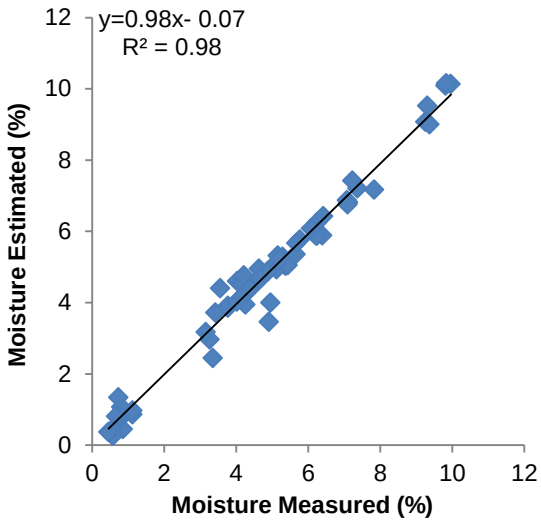
n: number of samples; x-Std: mean of standard values; x-NIR: mean of NIR values; SD: standard deviation of the mean difference; P-value: probability value at 95% confidence interval; SE mean: standard error of mean difference

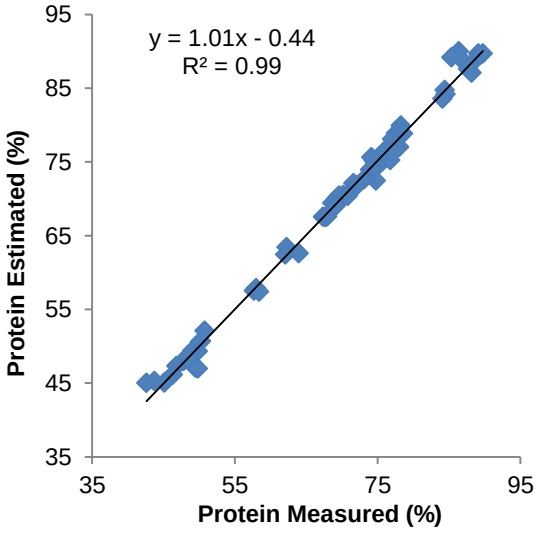
For example, in quantitative analysis, NIRS requires sufficient spectra to perform quantitative prediction of major and trace elements. This is to build the calibrations, which, together with advances in chemometric treatments, would improve available models in order to enhance their prediction capabilities. Another reason for low correlation of some minerals could be the evaluation method used through the cross-validation strategies, which basically consider

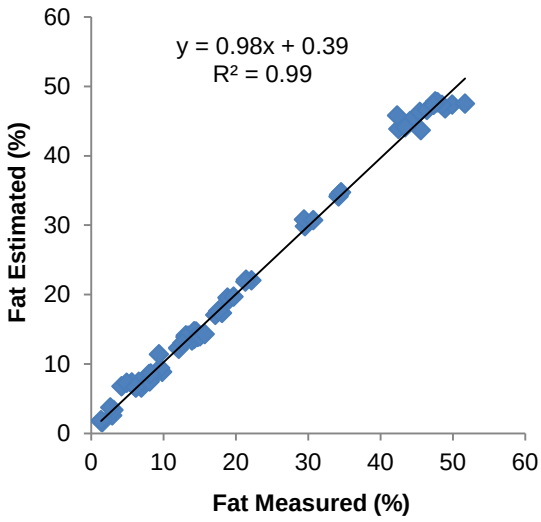
the whole population of samples used to build the model one by one in order to predict the concentration of each of the samples, which provides a poor evaluation of the ability of the developed model. Therefore, it is suggested to use external validation using an independent set of well-known concentration samples not employed during the calibration step which requires large set of samples (Garrigues and Guardia, 2015).

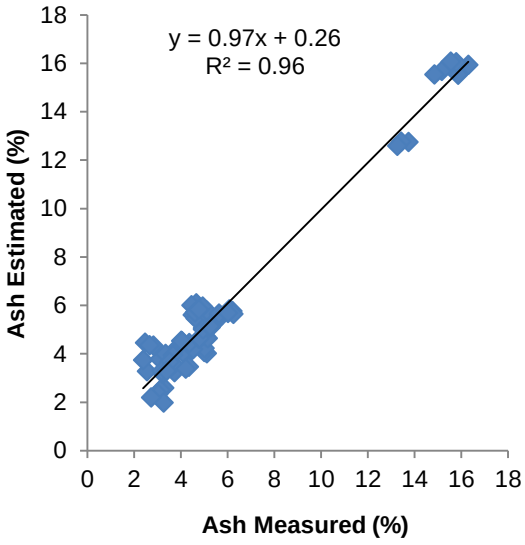
A paired t-test was also performed to check whether the NIR data and standard method values varied significantly. The predicted amount of all components from NIR and standard methods together with the prediction error are given in Table 5.3. There were no significant differences ($p>0.05$) between the values obtained by both methods.

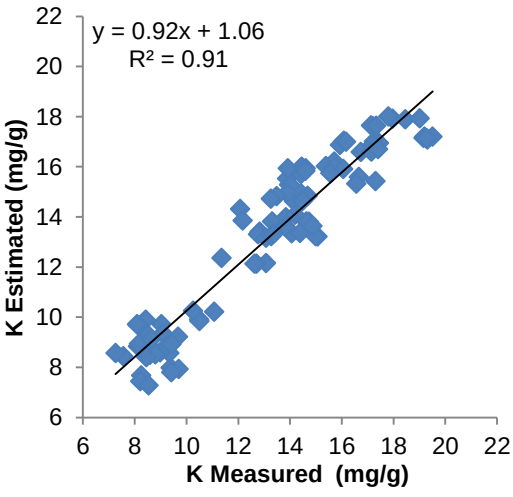
A linear regression equation (y=bias+ slope) was obtained by plotting spectra between the measured and estimated values obtained by standard method and NIR. The true concentration values of proximate and mineral compositions were plotted as a function of the predicted values, therefore the straight line represents the true values, the dots represent the predicted values. The plots scatter for the different components yielded straight line with highly correlation for the most components. However, a few components were yielded with lower correlation such as Na (Figure 5.3).

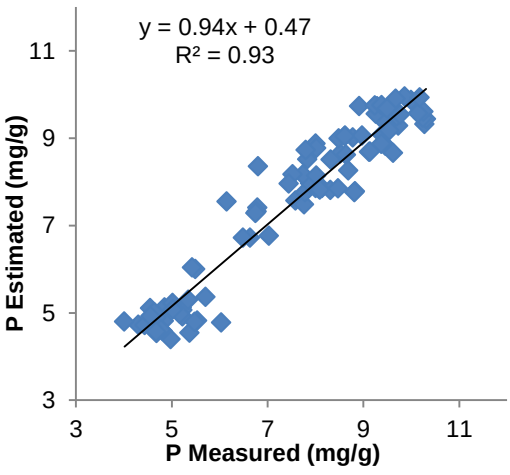

y=0.98x- 0.07
R² = 0.98
Moisture Estimated (%)
Moisture Measured (%)
0
2
4
6
8
10
12


y = 1.01x - 0.44
R² = 0.99
Protein Estimated (%)
Protein Measured (%)
35
45
55
65
75
85
95


y = 0.98x + 0.39
R² = 0.99
Fat Estimated (%)
Fat Measured (%)
0
10
20
30
40
50
60


y = 0.97x + 0.26
R² = 0.96
Ash Estimated (%)
Ash Measured (%)
0
2
4
6
8
10
12
14
16
18


y = 0.92x + 1.06
R² = 0.91
K Estimated (mg/g)
K Measured (mg/g)
6
8
10
12
14
16
18
20
22


y = 0.94x + 0.47
R² = 0.93
P Estimated (mg/g)
P Measured (mg/g)
3
5
7
9
11

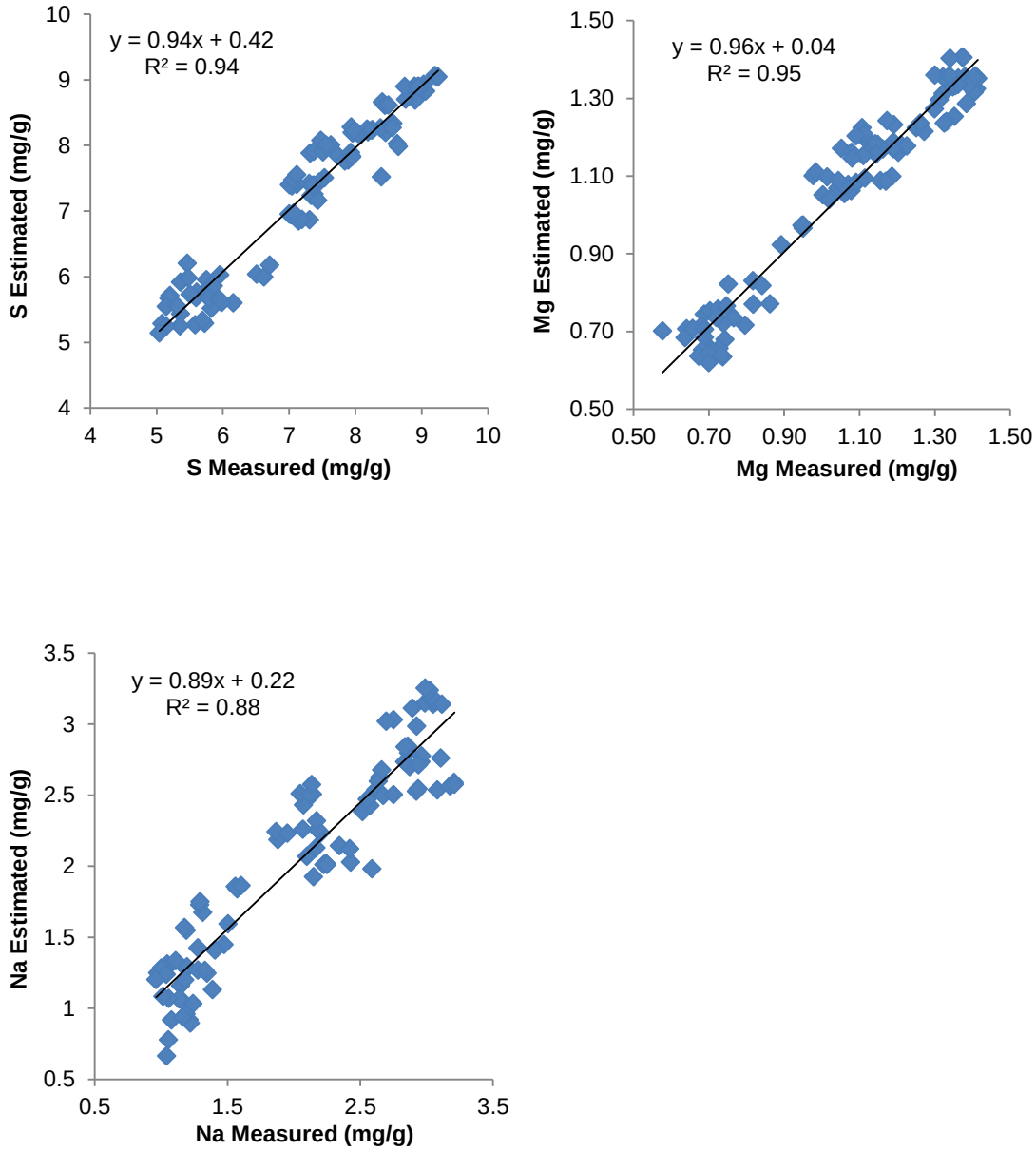


Figure 5.3: Linear correlation between the measured values obtained by standard methods and estimated values predicted by NIR for some chemical and minerals composition

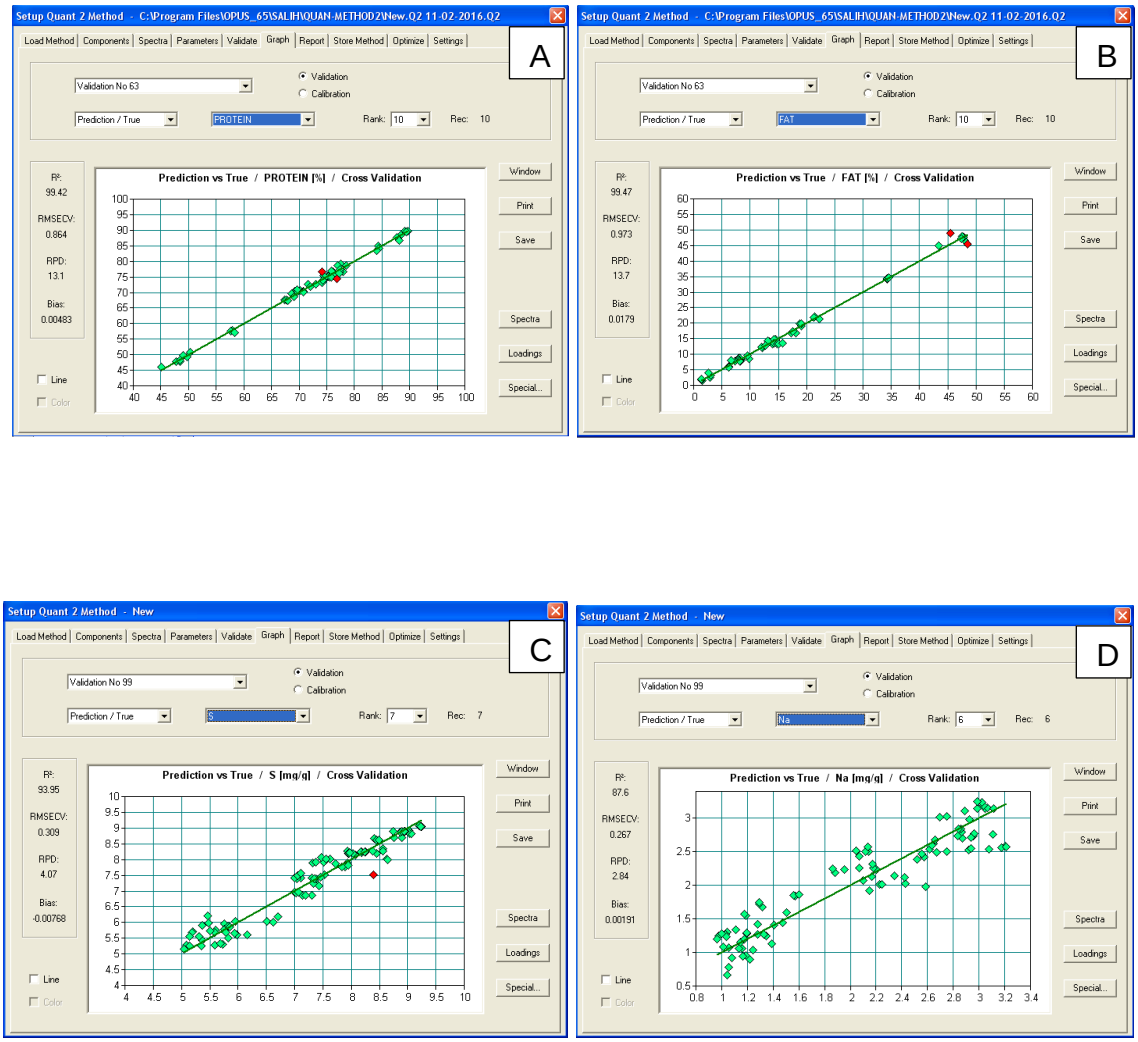


Figure 5.4: Examples of the windows which displays the correlation between the estimation values by NIR and values obtained by standard method when validating the calibration model, A (Moisture), B (Fat), C (S) and D (Na)

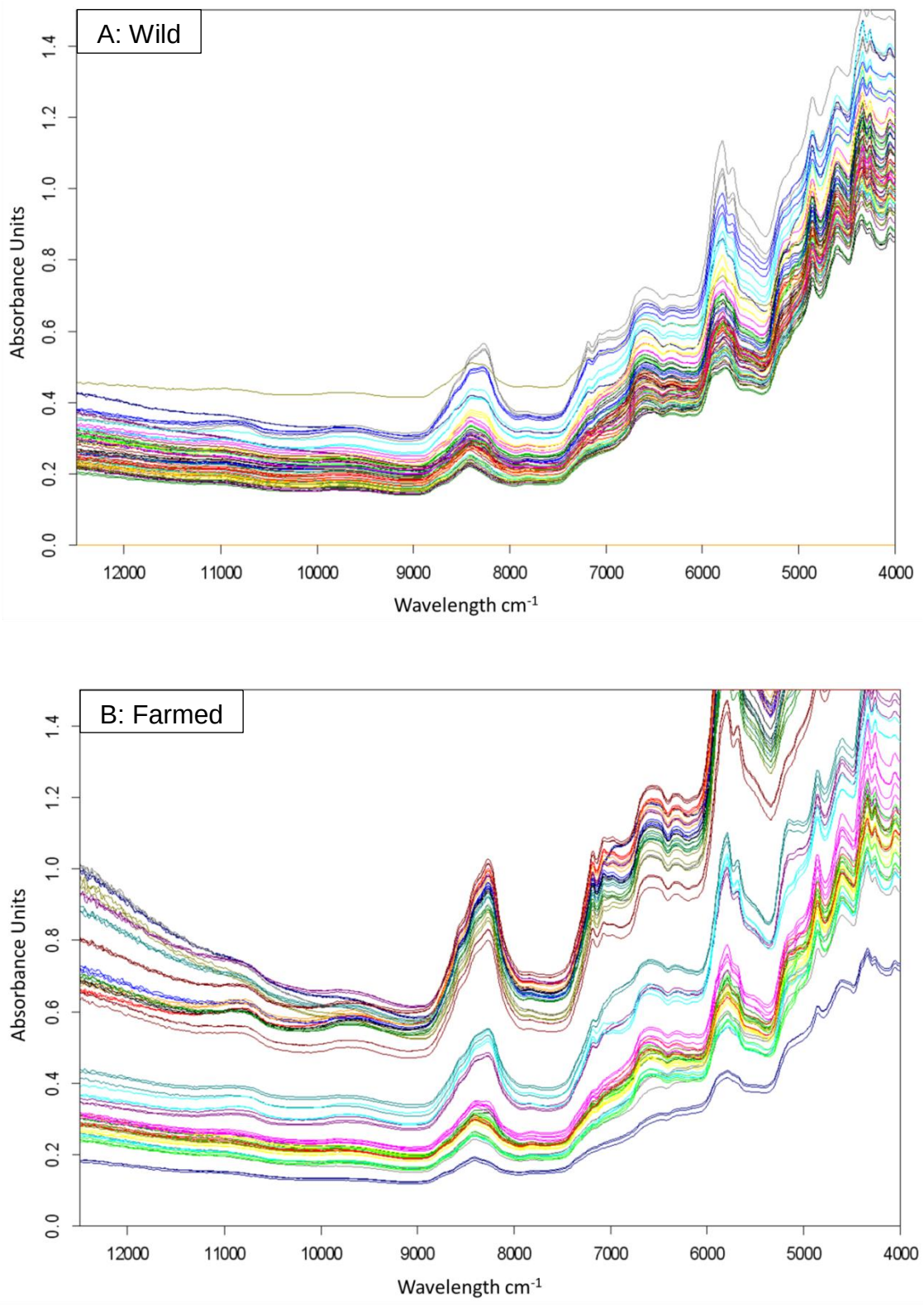


Figure 5.5: NIRS spectra corresponding to 56 samples (each in three replicates) of muscle fish of wild (A) and farmed (B) common carp over 12,500-4000cm^{-1} wavelength

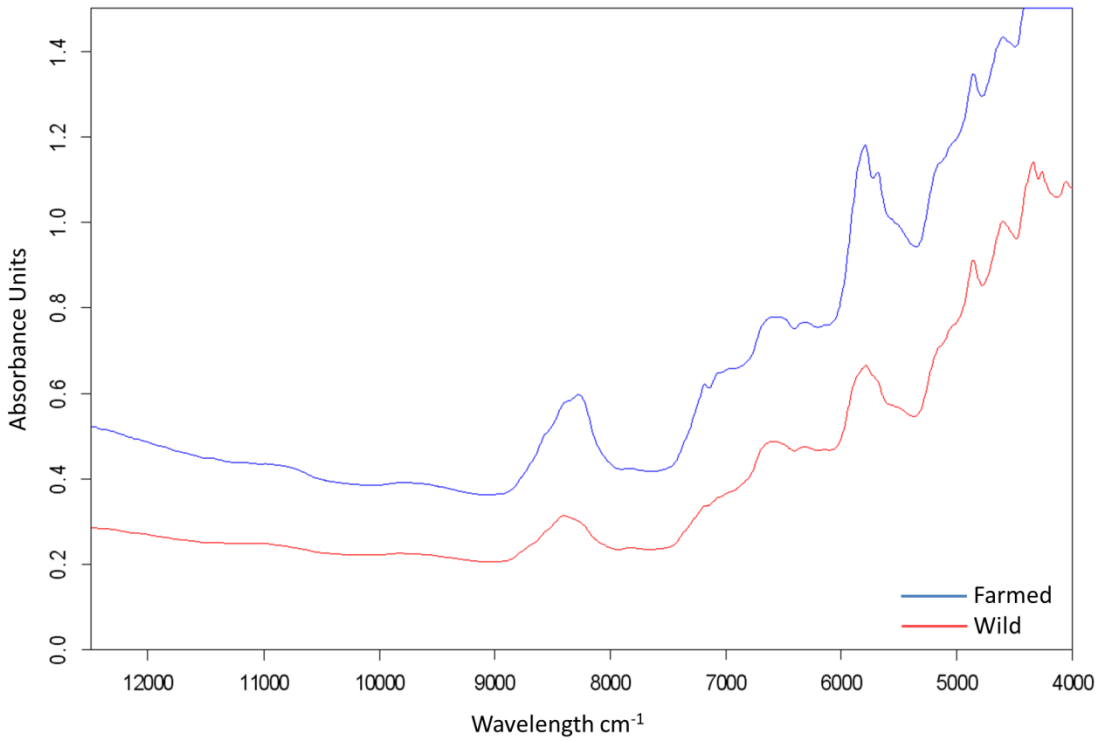


Figure 5.6: Mean raw spectra for the farmed and wild common carp

5.3.2 Authentication origin of production methods of common carp and species identity

5.3.2.1 Chemical composition

The fish quality differences between wild and farmed origin based on chemical composition has been widely reported (Grigorakis *et al.*, 2002, González *et al.*, 2006, Martínez *et al.*, 2010, Li *et al.*, 2011, Yeganeh *et al.*, 2012). Referring to existing literature, few studies addressed the comparative chemical composition in farmed and wild common carp.

The chemical composition of the freeze-dried muscle for each of *Cyprinus carpio* (wild and farmed), *Capoeta barroisi* and *Carassius auratus* measured by standard method and estimated by NIRS are presented in Table 5.4 and 5.5

respectively. Although there were no significant differences between wild and farmed common carp as well as between the three species, farmed samples were found to have higher fat and lower protein content than their wild counterparts. These results corroborate the findings of other studies on wild and farmed origin of common carp in Iran (Yeganeh *et al.*, 2012), wild and farmed origin of others species, such as sea bass (*Dicentrarchus labrax*) (Alasalvar *et al.*, 2002), yellow perch (*Perca flavescens*) (González *et al.*, 2006), sea bream (*Sparus aurata*) (Grigorakis *et al.*, 2002), turbot (*Psetta maxima*) (Martínez *et al.*, 2010) or as a general trend in fish origin (Nettleton and Exler, 1992).

However, protein is often considered to be a stable component in fish body in respect to diet and feeding level depending mainly on fish weight (Shearer, 1994). It usually increases with fish size, remaining stable after a certain size of fish reached (Shearer *et al.*, 1994).

The moisture content is usually inversely related to fat content (Grigorakis *et al.*, 2002), and moisture content was found in this study to be slightly higher in wild common carp compared to farmed samples, as observed in other studies (Alasalvar *et al.*, 2002, Yeganeh *et al.*, 2012). For the ash content of present study, no considerable differences were observed between wild and farmed common carp, similar to results found in sea bream (Grigorakis *et al.*, 2002).

However, wide variations on protein, fat and moisture concentrations were found (Table 5.3), and these variations were mostly observed within farmed and wild samples of common carp, especially in the fat and protein contents. This could be due to their differences in the feeding system (farmed samples) as they were purchased from different locations (farms), while the wild samples

may come from different environment conditions (lakes and rivers). Additionally, there could be a potential misclassification between wild and farmed common carp. For example, wild fish may be sold as farmed common carp for economic profit, which would affect the overall average of fat and protein levels. In addition, some experimental errors linked to the sampling preparation protocol may have contributed to these variations, as some fat could be lost during sample preparation, especially at the drying stage (homogeneous across samples due to nature of the fish), which eventually may influence the protein and moisture content as well. The differences in the level of fat and protein in muscle fish are linked to variations in nutrients availability between wild and farmed fish (Nettleton and Exler, 1992, González *et al.*, 2006), sensorial, chemical and physical properties (LINDSAY, 1980, Cox and Karahadian, 1998, Grigorakis *et al.*, 2003, Delwiche and Liggett, 2004, Mahboob *et al.*, 2009). The fish diet is one of the major factors that affects these properties (Cox and Karahadian, 1998, Alasalvar *et al.*, 2002).

For example, the chemical properties of wild fish are strongly influenced by the sea environmental conditions, which eventually determine the nutrient availability (Izquierdo *et al.*, 2003), whereas a feeding system with artificial diets mostly determines the nutrients and flesh composition in farmed fish (Periago *et al.*, 2005). Therefore, often farmed fish have a considerably higher fat content compared to wild fish. This is probably because cultured fish have considerably higher dietary fat level in the feed (~20%) and less activity (Alasalvar *et al.*, 2002).

A more homogeneous distribution among farmed products could be expected, as producers often aim to standardise their operations, and stocking brood is

often developed for consistent performance. However, as can be seen from NIR spectra (Figure 5.5B), the samples had more variation and the distributions of spectra were less homogeneous compared to wild spectra (Figure 5.5A). One reason explaining this variation among farmed samples could be the nature of the samples, which were purchased from different locations (section 5.2.1) and it would be expected that the growing conditions at different farms would vary, and also quite relevantly they would have different feeding systems, with diet being a major factor affecting composition. In contrast, wild samples were more homogeneous, and they were probably harvested from locations with similar environments (lakes and/ or rivers).

Another reason for the variation of compositional profile (Table 5.4) could be the drying methods applied to dry the samples.The samples were initially oven dried in KRI at different rates prior to transportation, and then upon arrival they were freeze dried in the UK, which possibly would have effects on the compositional profile. However, the samples were treated similarly to minimise any bias.

Table 5.4: Proximate composition of freeze-dried muscle fish measured by standard method and used for calibration model and validation (Mean±SD, n=3)

Component (%)	***Cyprinus carpio***		***Capoeta barroisi* (n=3)**	***Carassius auratus* (n=2)**
	Wild (n=7)	**Farmed (n=10)**		
Moisture	4.2±0.6	3.6±2.5	4.9±0.6	5.5±0.8
Protein	74.5±13.9	62.7±13.9	79.0±4.1	73.1±3.0
Fat	17.6±14.7	28.7±17.2	12.0±3.0	14.3±0.6
Ash	4.5±1.2	4.1±1.0	4.8±0.2	5.1±0.2

Table 5.5: Proximate composition of freeze-dried muscle fish estimated by NIRS (Mean±SD, n=3)

Component (%)	*Cyprinus carpio*		*Capoeta barroisi* (n=7)	*Carassius auratus* (n=3)
	Wild (n=19)	Farmed (n=27)		
Moisture	3.6±0.7	3.3±2.3	4.6±0.3	6.0±0.8
Protein	81.1±11.3	60.7±14.7	79.4±3.0	71.6±2.8
Fat	13.2±11.2	30.9±16.2	11.7±2.1	14.6±0.6
Ash	4.4±1.1	4.3±1.1	5.7±0.2	4.7±0.6

Table 5.6: Chemical comparison measured by standard methods and values estimated by NIRS after calibration model developed

Component (%)	Measured (n=22)			Estimated (n=56)			Deviation
	Range	Mean	SD	Range	Mean	SD	(mean)
Moisture	0.5- 7.8	4.1	1.8	0.1- 7.3	3.7	1.8	0.08
Protein	42.6- 89.7	69.6	13.9	38.6- 95.4	70.6	15.6	-1.68
Fat	1.4- 51.7	21.6	15.7	1.2- 49.2	21.6	15.8	-0.09
Ash	2.4- 6.3	4.4	1.0	1.5- 7.2	4.5	1.1	-0.12

In general, high correlation and lowest prediction errors (deviation) were obtained with the moisture, protein, fat and ash (Table 5.6). Therefore, NIR spectroscopy has been successfully used to predict the chemical composition of common carp and other two species (*Capoeta barroisi* and *Carassius auratus*). Furthermore, the successful applications of NIRS for predicting chemical

composition has been widely applied on various fish species, such as salmon, trout, and cod (Mathias *et al.*, 1987, Nortvedt *et al.*, 1998, Cozzolino *et al.*, 2002b).

Results of the principal component (PC) analysis of the wild and farmed fish on the basis of moisture, protein, fat and ash are plotted in Figure 5.7. Each data point is identified according to the origin authentication of wild and farmed common carp. Figure 5.7 shows the majority of farmed samples are on the left side of the plot and the majority of wild are on the right side. Therefore, for samples on the left side of the plot, that component has been more effective in separating wild from farmed common carp. However, there were some data values that would be out of the group, as a few of the wild carp samples were on the left side of the plot, while several farmed samples placed on the right side.

The variations within farmed samples were more likely to be observed as the farmed samples were present across the plot in both positive and negative sides. This can also be noticed from the raw spectral data obtained by NIRS (Figure 5.5B). The most likely reasons for this misclassification could be due to the growing conditions of collected farmed fish from different farms and/ or locations with different feeding systems across the region as well as the possibility of mislabelling between farmed common carp and wild.

Regarding the coefficient of the PC that has been produced for each variable, the two first PCs accounted for 88% of the total variability. The total weight on the first component (explaining 72% of the variability) where the majority of wild common carp samples were identified was the combination of all variables. For

example, moisture, protein and ash dominated the positive side of PC1, while the fat content was the only variable on the negative side of PC1 (Table 5.7). In the second PC, ash had the highest scores on the positive side in combination with fat (Table 5.7) where the origin authentication of farmed common carp was discriminated from wild samples (Figure 5.7), while the negative side of PC2 are the combination of moisture and protein. Therefore, it can be concluded that the concentration of ash was the most effective variable for discriminating farmed common carp from wild.

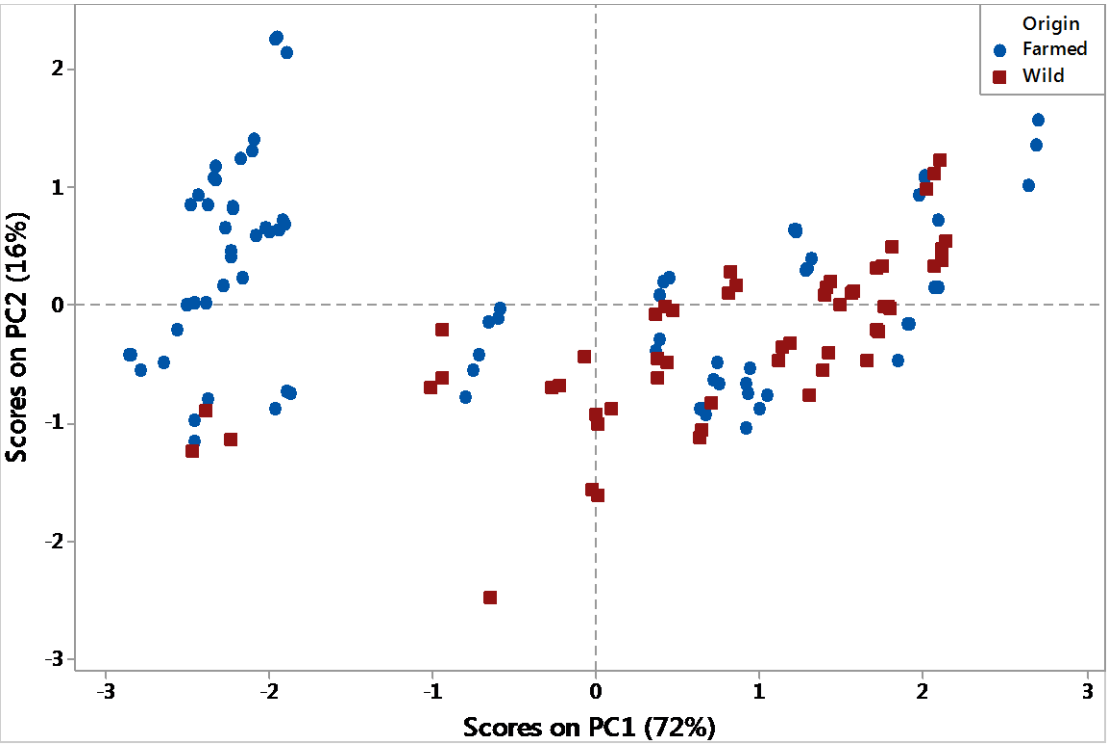


Figure 5.7: Principal component analysis score plot of the estimated (NIR) chemical composition of wild and farmed common carp (n=46)

Table 5.7: Eigenvalue, proportions of each component and the weight of each variable within each component for farmed and wild origin of common carp

Components	Proportion (%)	Eigenvalue	Weight of variables			
			Moisture	Protein	Fat	Ash
PC1	72	2.88	0.47	0.54	-0.57	0.41
PC2	16	0.64	-0.26	-0.28	0.17	0.91
PC3	12	0.48	0.84	-0.49	0.25	0.04
PC4	0.00	0.004	-0.13	-0.63	-0.76	-0.09

For classification of the three species, samples of *Cyprinus carpio* (wild and farm) were distributed over the first and second PC, while the samples of both *Capoeta barroisi* and *Carassius auratus* were all located on the right side of the plot (Figure 5.8). Therefore, the first PC (right side of the plot) has been very effective in separating the three fish species compared to the PC2.

In general, both PC1 and PC2 explained 88% of the total variability. The PC1 (explaining 73% of the total variability) consists of the combination of moisture, protein and ash on the positive side, while the fat content was on the negative side of plot line (Table 5.8). In the second PC (explaining 15% of the variability) with only *Cyprinus carpio* samples, ash was the dominating variable on the positive side (Table 5.10), while on the negative side of PC2 was the combination of moisture and protein. Again, ash was the main variable differentiating *Cyprinus carpio* from other two species, fat for *Carassius auratus*

and a combination of moisture, protein and ash discriminated *Capoeta barroisi* from other two species.

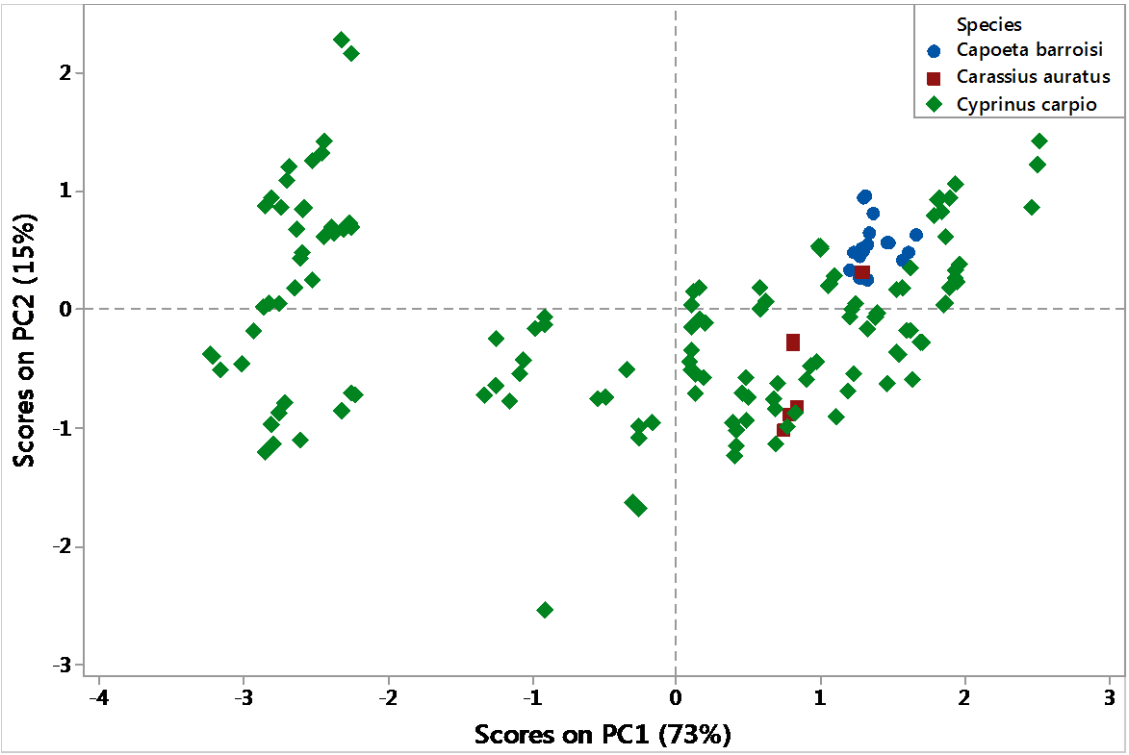


Figure 5.8: Principal component analysis score plot of the estimated (NIR) chemical composition of the three fish species (n=56)

Table 5.8: Eigenvalue, proportions of each component and the weight of each variable within each component for species origin identity

Components	Proportion (%)	Eigenvalue	Weight of variables			
			Moisture	Protein	Fat	Ash
PC1	73	2.92	0.46	0.53	-0.57	0.42
PC2	15	0.59	-0.26	-0.29	0.19	0.90
PC3	12	0.49	0.84	-0.50	0.23	0.03
PC4	0.00	0.004	-0.15	-0.62	-0.76	-0.08

5.3.2.2 Minerals composition

In the present study, ICP-MS and ICP-OES techniques were used for minerals composition, and the ICP-MS subjected to validation process with certified values. The percentage of the certified values recovered for all analysed micro-minerals for ICP-MS was 87-106% as shown in Table 5.2. The results of micro-mineral recovery ensure the validity of the method (ICP-MS) used in this study. The reason for not validating ICP-OES method with certified values was due to the unavailability of the certified values of some macro-minerals analysed in this study with ICP-OES.

Table 5.9 shows the mean concentration and the SD values of all measured mineral composition of muscle fish according to the origin of production method of Cyprinus carpio (wild and farmed), and species identity (Cyprinus carpio, *Capoeta barroisi* and *Carassius auratus*) obtained by the chemical reference methods (ICP-MS and ICP-OES).

In general, all analysed minerals were determined using ICP-OES for macro-minerals and ICP-MS for micro-minerals, except the concentration of Hg was not detected with ICP-MS due to their low concentration. It is therefore concluded that this instrument is not sensitive enough to determine this toxic element.

Potassium (K) was the most abundant mineral compound found in muscle fish of the three species. The concentration of K with P and S showed they were the predominant minerals and constituted about 85% of the total minerals analysed in both of wild and farmed common carp, and in other two species. The mineral contents of most analysed samples were not affected significantly ($p<0.05$) by growing conditions differing between wild and farmed treatments.

However, the general accumulation patterns of analysed minerals (K, P, S, Na, Ca, Mg, Zn, Fe, and Se) tend to have slightly higher (but not significant) concentrations in wild common carp, while the concentrations of Cr, Cu, and Mn were slightly higher in farmed fish, and for others minerals were negligible. In a similar previous study in comparison of trace element concentration in wild and cultured common carp in Japan, no significant differences ($p>0.05$) were found in concentrations of Zn, Fe, Se, Cu, Mn, Pb, Cr, Co and Cd between farmed and wild common carp (Alam *et al.*, 2002).

The significant ($p<0.05$) differences in the present study only appeared for arsenic (As) and selenium (Se), where wild common carp had higher concentrations (0.23 and 1.47 µg/g respectively) than farmed samples (0.03 and 0.53 µg/g respectively). In contrast, Alam *et al.* (2002) found that farmed

common carp had significantly ($p<0.05$) higher concentrations of arsenic (As) (0.179 µg/g) compared to wild fish (0.095 µg/g).

In terms of differences between the three species, most of the detected minerals in muscle fish of *Capoeta barroisi* were of slightly higher concentration when compared to the other two species, while Na, Mn and Co were at significantly higher levels in *Capoeta barroisi*. In contrast, arsenic was found at significantly higher levels in *Carassius auratus* than in the other two species.

The concentration of minerals in fish species is generally influenced by a number of factors such as seasonal, biological differences (species, size, age, sex, and sexual maturity), nutrient source, environment (water chemistry, salinity, temperature, and contaminants), and also the method of food processing (Lal, 1995, Carvalho *et al.*, 2005).

Table 5.9: Minerals composition of *Cyprinus carpio* fish (dry weight) according to production origin and species using ICP-MS and ICP-OES (n=56)

Minerals (mg/g)	Origin		Species		
	Wild (n=19)	**Farm (n=27)**	**Cyprinus carpio (n=46)**	***Capoeta barroisi* (n=7)**	***Carassius auratus* (n=3)**
Macro-Minerals					
K	14.85±3.01	11.26±3.25	12.74±3.61	14.05±0.57	13.24±1.25
P	8.39±1.61	6.44±1.93	7.24±2.04	8.14± 0.58	7.39±0.73
S	8.13±0.93	6.43±1.35	7.13±1.46	8.03±0.59	8.27±0.35
Na	2.29±0.68	1.58±0.53	1.88±0.69^{a}	2.95±0.33^{b}	1.72±0.50^{a}
Ca	2.12±0.82	1.44±0.68	1.72±0.81	2.24±0.79	1.43±0.30
Mg	1.18±0.24	0.87±0.21	0.99±0.27	1.14±0.05	1.11±0.05
Zn	0.04±0.01	0.03±0.01	0.03±0.01	0.03±0.00	0.06±0.03
Fe	0.04±0.01	0.03±0.01	0.03±0.01	0.03±0.01	0.05±0.01
Micro-Minerals					
Cu	3.72±1.64	3.97±1.72	3.86±1.69	3.19±0.79	4.73±0.96
Se	1.47±0.61^{A}	0.53±0.28^{B}	0.92±0.73	1.68±0.94	1.02±0.19
Mn	0.82±0.29	0.87±0.22	0.84±0.26^{ab}	1.72±0.75^{a}	0.60±0.08^{b}
Cr	0.27±0.09	0.49±0.25	0.40±0.30	0.22±0.08	0.36±0.32
As	0.23±0.16^{A}	0.03±0.03^{B}	0.11±0.11^{a}	0.29±0.07^{a}	0.67±0.17^{b}
Pb	0.09±0.03	0.10±0.04	0.09±0.04	0.08±0.02	0.08±0.02
Co	0.03±0.01	0.04±0.03	0.03±0.02^{ab}	0.06±0.02^{a}	0.02±0.01^{b}
Cd	0.004±0.004	0.004±0.002	0.004±0.003	0.006±0.005	0.006±0.002

$^{A\text{-}B \text{ and } a\text{-}c}$ Mean±SD (n=3) with different letters in the same row (each of origin and species) are significant different at $p < 0.05$

Table 5.10: Chemical comparison between measured minerals and all quantifying (predicted) of the unknown of minerals compositions of muscle fish after calibration model developed

Min*	Measured values (n=33)			Estimated values (n=56)			Deviation (mean)
	Range	Mean	SD	Range	Mean	SD	
K	7.26- 19.51	13.34	3.44	6.29 - 19.51	12.93	3.32	0.41
P	4.01- 10.31	7.52	1.94	3.62 - 12.22	7.36	1.89	0.16
S	5.04- 9.25	7.27	1.26	4.04 - 9.42	7.30	1.39	-0.03
Na	0.96- 3.21	2.05	0.76	0.83 - 4.23	2.00	0.74	0.05
Mg	0.78- 1.41	1.05	0.25	0.54 - 1.52	1.02	0.25	0.03
Ca	0.69- 3.41	1.79	0.75	0.46 - 4.16	1.77	0.81	0.02
Zn	0.02- 0.06	0.03	0.01	0.017 - 0.077	0.034	0.012	0.00
Fe	0.01- 0.05	0.03	0.01	0.013 - 0.065	0.034	0.011	0.00
Cu	1.41- 11.91	3.57	1.47	1.410 - 11.908	3.826	1.597	-0.26
Se	0.20- 3.87	1.10	0.88	0.173 - 3.868	1.017	0.779	0.08
Mn	0.44- 3.13	0.99	0.44	0.370 - 3.133	0.943	0.460	0.05
Cr	0.10- 4.69	0.36	0.46	0.102 - 4.695	0.374	0.472	0.01
As	0.002- 0.53	0.17	0.17	0.002 - 0.912	0.165	0.193	0.01
Pb	0.04- 0.25	0.09	0.03	0.043 - 0.246	0.092	0.036	0.00
Co	0.10-0.17	0.04	0.02	0.008 - 0.174	0.035	0.025	0.00
Cd	0.001-0.040	0.004	0.004	0.001 - 0.039	0.004	0.004	0.00

*****Min=minerals

Despite many studies published on mineral accumulation in common carp tissue (Goldstein and DeWeese, 1999, Čelechovská *et al.*, 2007, Qin *et al.*, 2015), few studies used element concentrations to discriminate between wild and farmed common carp (Alam *et al.*, 2002). The potential of using NIRS for quantifying the macro-minerals for discrimination of origin of production method and classification of species types was analysed with the PCA technique to better visualise (Figure 5.9 and 5.10). Good correlations were obtained for most of the macro-mineral concentrations (Figure 5.3 and Table 5.3). However, very low correlations were obtained for all micro-minerals, indicating that NIRS was not successful.

The most studied elements in foods including fish using NIRS were Ca, K, Mg, S, Fe and Zn (Garrigues and Guardia, 2015). Figure 5.9 shows the plot that taking part mineral concentration according to the origin of the production method as variables resulted in two principal component models describing about 84% of the total data variability. In particular, PC1 explained about 76% of the variability and PC2 explained 8%. The score plot of the first two principal components indicated the grouping of production method (wild and farmed) of common carp. In general, wild samples were mostly placed on the plot, while the majority of farmed common carp were placed on CP2. Furthermore, the positive side of PC2 was more effective for differentiating farmed fish from wild.

However, many data values have been misclassified between both PCs as explained earlier with chemical composition. The most likely reason for this misclassification could be due to the mislabelling of farmed and wild common carp or farmed samples sold as wild for economic gain. Another factor that should be taken into consideration is the potential variability within wild and

farmed common carp collected from different rearing systems, as shown in the raw spectra graph particularly for farmed common carp (Figure 5.5)

The total weight of each mineral contributing to the discrimination of origin was described in Table 5.11. For example, all minerals were located on the positive side of PC1. In the second PC, Fe and Na were dominant variables on the positive side where the farmed common carp more effectively differentiated from wild samples (Figure 5.9), while the negative side of PC2 were mostly the combination of K, Mg, S and P. Therefore, it can be concluded that the concentration of Fe and Na were the more effective variables for discriminating farmed common carp from wild.

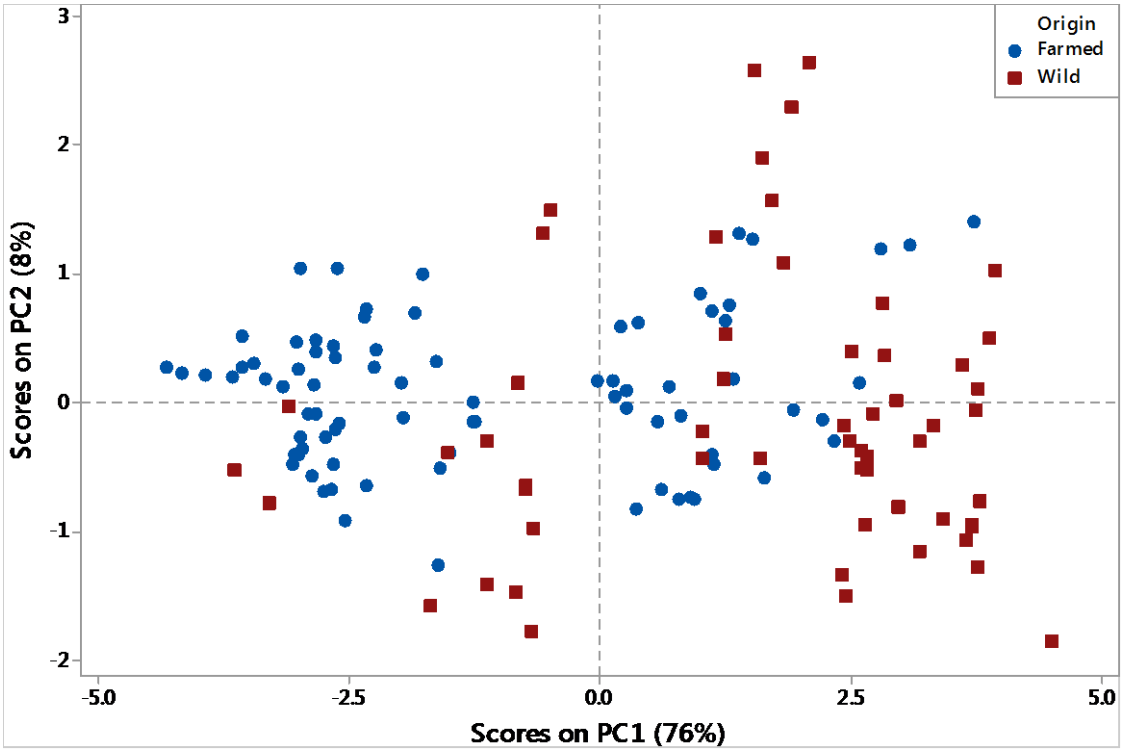


Figure 5.9: Principal component analysis score plot of the estimated (NIR) minerals concentration of the wild and farmed common carp (n=46)

Table 5.11: Eigenvalue, proportions of each component and the weight of each variable within each component for origin of production method

Variables	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
Proportion (%)	76	8	7	4.2	2.5	1.5	0.5	0.3
Eigenvalue	6.05	0.67	0.56	0.33	0.21	0.12	0.04	0.02
K	0.38	-0.30	0.13	-0.23	0.27	-0.27	-0.43	-0.61
P	0.39	-0.20	-0.10	-0.30	0.19	-0.24	-0.19	0.77
S	0.37	-0.5	0.31	-0.11	-0.50	0.66	-0.26	0.04
Na	0.35	0.34	0.29	0.29	-0.48	-0.60	-0.09	0.00
Ca	0.30	0.11	-0.89	0.03	-0.28	0.03	-0.11	-013
Mg	0.38	-0.31	0.00	-022	-0.00	0.07	0.83	-0.14
Zn	0.35	-0.17	0.01	0.82	0.38	0.20	-0.10	0.07
Fe	0.29	0.79	0.05	-0.21	0.44	0.20	0.08	-0.06

Consistent with the classification of the three species based on proximate composition explained earlier, mineral concentrationse also effectively classified the three species. Although the samples of *Cyprinus carpio* (wild and farm) were distributed over the first and second PC, all samples of *Capoeta barroisi* were located on the negative side of PC1, while all samples of *Carassius auratus* were located on the positive side on PC1 (Figure 5.10). Therefore, the first PC (explaining 69% of the total variability) was very effective in separating the three fish species compared to the PC2 (explaining only 10 of the variability). Furthermore, in the PC1, all the minerals were located on the positive side. Whereas, in the PC2 Fe and Zn were the predominant variable on the positive side of line and both Ca and Na were the major variables on negative side.

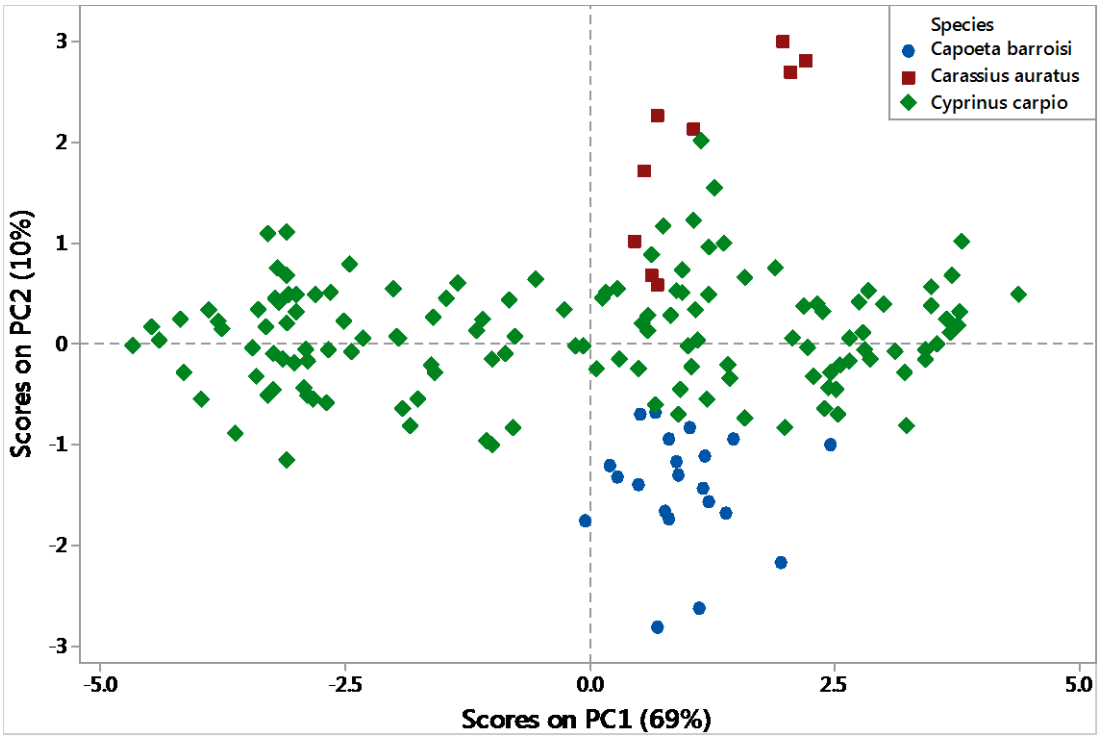


Figure 5.10: Principal component analysis score plot of the estimated (NIR) minerals concentration of the three fish species (n=56)

5.3.3 Assessing toxic element concentrations in fish muscles

Studies on essential and toxic mineral content in foodstuffs have developed extensively in recent decades. Evaluation of the potential health benefits and risks of fish consumption has been controversial. Although fish are important sources of high-quality protein, minerals and essential fatty acids such as omega-3, conversely, fish could be a major vector for toxic substances (Marcovecchio *et al.*, 2015).

Table 5.12: Eigenvalue, proportions of each component and the weight of each variable within each component for species origin identity

Variables	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
Proportion (%)	69	10	7.7	6.7	3.9	2	0.5	0.3
Eigenvalue	5.54	0.78	0.62	0.54	0.31	0.16	0.04	0.02
K	0.40	-0.08	-0.26	-0.10	0.34	-0.31	-0.46	-0.58
P	0.41	-0.14	-0.04	-0.13	0.36	-0.22	-0.14	0.78
S	0.38	-0.04	-0.25	0.28	-0.11	0.79	-0.28	0.06
Na	0.33	-0.31	0.11	0.65	-0.45	-0.39	0.08	-0.00
Ca	0.29	-0.34	-0.70	-0.46	-0.21	0.18	-0.10	-0.13
Mg	0.41	-0.14	-0.21	-0.13	0.19	0.11	0.82	-0.18
Zn	0.31	0.52	-0.26	-0.39	-0.61	-0.18	-0.01	0.07
Fe	0.27	0.69	0.51	0.31	0.30	-0.00	0.08	-0.07

Minerals can be classified as essential elements because of their important role in biological systems, while some trace elements, such As, Cd, and Pb belong to non-essential and toxic elements and do not play any metabolic function. Instead, they can be harmful for human consumption, even at low concentration, when ingested over a long time period (Fallah *et al.*, 2011). Therefore, fish producers and general public have concerns over fish contamination (Chapman *et al.*, 1996).

According to the European Commission (EC), the permissible limits for Cd and Pb in fish for human consumption are 0.05 and 0.3 µg/g of wet weight respectively (European Commision, 2006). The concentrations of all heavy and toxic elements examined in this study were lower than the legislated limits. For example, the concentrations of Cd (0.004 µg/g) and Pb (0.10 µg/g) in dried muscle of farmed and wild common carp (5.9) were lower than permissible limits.

The values of the present study were also lower compared to the same fish species reported in Turkey (Özparlak *et al.*, 2012) with the concentrations of Pb and Cd were 2.84 and 2.17 mg/kg respectively on dry weight basis. Furthermore, it was also much lower than the concentration of each of Pb, Cd and As determined in common carp in Northeast China (Qin *et al.*, 2015).

Fish could be a major source of total arsenic exposure for humans. Toxicity of different As species in marine samples is highly dependent on their oxidation states and chemical forms (Sadee *et al.*, 2015). Arsenic concentration in the muscle tissues in this study was significantly higher in wild (0.23µg/g) compared to farmed samples (0.03 µg/g), which was lower than the concentration of arsenic reported in common carp from Kasumigaura Lake in Japan of wild (0.095 µg/g) and farmed (0.179 µg/g) in wet weight (Alam *et al.*, 2002). Furthermore, Goldstein and DeWeese (1999) found that the concentration of arsenic in muscle of common carp (0.24 µg/g) was much lower than in liver (0.40 µg/g), and whole body (1.18 µg/g) of dry weight.

Although only muscle fish were studied here, previous reports indicated that lower mineral levels were accumulated in fish muscle than in other organs.

Alam *et al.* (2002) found that for minerals including toxic elements (Pb, As, and Cd) concentrations were lower in muscle than in other organs such as liver, intestine, kidney, and gonads of common carp. Furthermore, Fallah *et al.* (2011) also found a significantly higher ($p<0.05$) concentrations of As, Cd, Pb, Se and Zn in liver compared to muscle of wild and farmed rainbow trout.

Essential trace elements such as Cr, Mn, Co, Cu, Zn and Se can also produce toxicity but only at high concentrations (when taken in excessive amounts) (Marcovecchio *et al.*, 2015).

5.4 Conclusion

The differences on overall quality of wild and farmed origin of fish species are always a subject of discussion to verify the differences and avoid mislabelling. Proximate composition and mineral levels are important constituents that can be used for these purposes.

This study revealed that the analysed components (chemical composition and minerals) of muscle fish predicted by NIRS had a strong correlation with the values obtained by standard methods, whilst for macro-minerals the correlation was also good to predict the mineral compositions. These results, together with the parameters obtained by chemometric analysis, confirmed that NIRS was a promising method that can provide accurate predictions for proximate composition and some macro-minerals on muscle fish as an effective alternative to traditional standard chemical methods, being a rapid and non-destructive assessment for discriminating between wild and farmed common carp.

However, the trends of prediction models for micro- minerals were not satisfactorily accurate. Regarding the PCA analysis, it did not clearly discriminate between wild and farmed fish, neither for proximate composition, nor for mineral composition. The slight differences in chemical composition of muscle fish of wild and farmed common carp measured using NIRS seemed to be an interesting subject to investigate further with a larger calibration data set, in particular to address the low predictability of micro-minerals.

Our results suggested that the wild and farmed common carp reared in Kurdistan are not contaminated with toxic trace elements. Despite their dietary

differences, the wild and farmed common carp had similar accumulation levels except for a few minerals, with negligible differences in minerals concentrations. At the levels detected, minerals should pose no health problems for consumers of these fish.

The predictive model for mineral composition showed good accuracy for some minerals, but attention should be paid to whether larger sample sets will improve them sufficiently to enable more precise prediction of the concentrations of each mineral. Further work would be needed to optimize the accuracy of the predicted model. Some toxic trace minerals such as Arsenic (As) can accumulate in different forms or species of fish tissue as organic or inorganic. The separation and quantification of different arsenic species with the combination of coupled HPLC and ICP-MS will be necessary to establish likely toxicity.

Chapter 6

General discussion and conclusions

6.1 General discussion and conclusions

The current food supply chain networks continue to grow in scale and complexity, and deliberate food fraud, driven by the prospect of economic gain, is an emerging risk. Determining the authenticity of foods can prevent false description, substitution of cheaper ingredients, and adulteration, as well as incorrect origin labelling.

Issues of food authenticity and frauds include mislabelling, substitution, adulteration, misrepresentation of geographical and production origin, misleading food composition or use of unexpected, undeclared, or not-allowed ingredients as extenders or additives, and technological processes such as freezing or heating. Subsequently, several economic issues arise, including cheating consumers, unfair competition, non-compliance with the standard requirements, damage to perception of products, and economic losses to production sectors or regions due to the lost trade.

Therefore, article 8 of Regulation 178/2002 deals with the protection of consumers' interests and requires food law that provide a basis for consumers to make informed choices in relation to the foods they eat. Furthermore, food law must aim to prevent fraudulent or deceptive practices, the adulteration of food, and any other practices which may mislead the consumer (European Commission, 2002). Ensuring the safety, quality, and authenticity of food along the supply chain is a major challenge (Aung and Chang, 2014, Porcari *et al.*, 2016), and governments around the globe are intensifying their efforts to understand and improve the food quality systems (Elliott, 2014). Dedicated organisations, task forces, and food quality standards have been laid down by many countries to tackle the food authenticity issues. More rapid, reliable, and

affordable techniques to systematically assess the vulnerability of fraud in food supply chains would be valuable.

Concerns over ongoing food authenticity issues have reduced consumers' confidence. Recent cases of food fraud have received considerable media attention, including notable examples such as the horsemeat scandal in Europe, fish mislabelling in USA and melamine in milk in China. Generally different scandals expose different dimensions of trust in food safety, nutrition, economic value, quality, and environmental and animal welfare ethics (Kjaernes *et al.*, 2007).

There is a lack of research exploring consumers' trust on the issues of food authenticity of animal products within KRI and the availability of analytical methods for quality control and food safety. One of the purposes of the current thesis was to obtain an overview of the understanding of consumers' perception and the state of the art of technical issues of food authenticity of animal products as discussed in the literature review. The study included tasks aiming to identify the potential issues of the food authenticity of animal products.

These issues were first explored and investigated using qualitative and quantitative tools, focusing on how consumers in perceive food authenticity issues and their main concerns regarding food of animal origin. Several fraud detection approaches were used to monitor how well fraud can be detected with current available techniques. A line of enquiry was the influence of cultural and geographical background, with panels including Middle Eastern participants required in the UK, and research tools applied in KRI.

One of the main reasons for selecting the kebab meat products as a case study in the first two experiments (Chapters 2 and 3) were due to many issues

reported by the LACORS on the doner kebab using a survey by 76 individual councils in the UK. The survey found remarkably high levels of fat, salt and calories in doner kebab that contains more than 1000 calories, which is about half of the GDA. In addition, there were no clear details of the meat species and the quantities of each meat species in doner kebab due to the lack of requirements for catering establishments to provide details of the meat content when the product is sold unpacked to the final consumer (LACORS, 2009).

However, because FG discussions were based on small and not representative samples, FG should therefore not be used as the only empirical evidence to support the final conclusions (Morgan, 1997), and the results of the quantitative WBS could support the conclusions and add more robustness to the overall obtained data. The obtained data (Chapter 2) indicated that consumers' attitude towards kebab meat products were varying depending on the sociodemographic factors, such as cultural groups, gender, and age groups. These variations were more obvious within cultural groups between British/ European and Middle Eastern background, including Kurdish, regarding frequency of eating, preferences and trust of the types of fast foods, meat species and levels of meat species in KMP.

This probably was because consumers often demand the same products and services at their new homes when they change their place of residence (Sirkeci, 2016). Therefore, Middle Eastern cultural groups, including Kurdish, tend to eat KMP more frequently and trust in KMP compared to other groups. In contrast, a sausage for example was more in favour within British/ European respondents.

From the questionnaires and FG studies (Chapter 2), respondents were asked several questions regarding the composition of KMP, including the fat levels and the added new ingredients such as inulin fibre. In both tools, respondents expressed their concerns about the high level of fat content of KMP. Generally, the idea of adding the inulin as functional ingredients for replacing fat in KMP was seen as a positive effect.

However, some participants did not agree with adding any new ingredients, while those who agreed wondered if inulin could reduce fat content and make the product healthier. Furthermore, as explained earlier, the excessive consumption of processed meat products high in fat and deficient in fibre, such as doner kebab, would add to the negative perception and may be linked to the development of some chronic diseases. Therefore, an experiment was designed to reformulate the doner kebab with inulin as a fat replacer. The aim was firstly to assess the effect of inulin as a fat replacer using physicochemical tests, secondly to examine consumer acceptability of the new product using sensory evaluation test, and finally to apply an enzymatic assay to assess the identification limits of inulin in meat products for labelling purposes (Chapter 3).

Physiochemical, texture analysis, cooking characteristics, microstructure and sensory evaluation tests are often used for assessing eating quality of a product development. The results of these tests provided clear evidence that the quality attributes of doner kebabs were maintained while reducing fat content by 50% of the original level, by using inulin.

The findings from this study showed that the consumer panels had a preference towards the product containing added inulin, especially from JA compared to

the control product, for the overall appearance, colour, juiciness, fattiness and overall acceptability. These results were positive attributes that modification of kebab meat products could be made through the inclusion of different types, levels and forms of inulin with desirable characteristics. This positive trend has been previously reported by several researchers, as explained in the literature review, for different meat products where inulin was added as fat substitution (Mendoza *et al.*, 2001, Selgas *et al.*, 2005, Menegas, 2013, Keenan *et al.*, 2014).

However, in order to compare the prepared doner kebab in resent study in regards to its compositional and cooking characteristics to a reference sample, three commercial doker kebabs were analysed as references. The significant data on cooking characteristics and the less variations on its composition compared to the prepared model system could be due to the preparation method followed by manufacturer to reduce the cost and the ingredients used that may help to hold the structure and eventually increase the cooking yield, and retain water and fat. Whereas, the premium goal with model system of doner kebab prepared in this study was to exclusively see the effect of inulin without having any other fibres.

Furthermore, the tubers of JA harvested in KRI were studied as probiotic supplement in feed for growth performance of broiler chickens and their effect on overall meat quality attributes (Akoy, 2015). However, its application in food products has not been investigated yet in KRI, and therefore preparing flours from tubers of JA could be an appropriate technology in an area of the world where specialist food ingredients appear to be costly due to currency differences and the costs of importing.

Additionally, the evaluation of the applied enzymatic assay for inulin identification was performed. The assay was sensitive and reliable enough to detect inulin down to 0.5% in meat products. Therefore, it would be necessary to use a sensitive and accurate method to detect inulin at lower levels below 0.1% as proposed for further study. On the other hand, as discussed in the literature review, foods from animal origin, especially fish, were accounted as the top mislabelled food especially in the US. Therefore, despite having a legal requirement (EU directive EC/2065/2001) for clear labelling information including fish species, geographical origin, and production method of fish (Jacquet and Pauly, 2008), seafood fraud as a global problem can take different forms, including false labelling, species substitution and false origin authentication (i.e. wild or farmed fish) (Golden and Warner, 2014).

Although seafood is considered as an important source of protein, and thus an important food for many people, the price differences between farmed and wild fish and between different fish species make mislabelling and fraud profitable and labelling information may not be always correct (FSA, 2007). Moreover, farmed and wild fish may contain different hazads which may be subject to different regulations and analytical controls (Martinez *et al.*, 2010).

The main contributions to seafood mislabelling (Miller and Mariani, 2010, Hanner *et al.*, 2011) are the lack of regulation and labelling enforcement, a situation consistent with observation in KRI during the sample collection period.

For the aforementioned reasons the third and fourth experiments (Chapters 4 and 5) were carried out based on the local (majority carp) and several imported fish samples collected from KRI and analysed at Plymouth University. Molecular DNA based methods and NIR spectroscopy were demonstrated as a potential

and rapid quality monitoring method for fish species identification and origin authentication of production method. This was due to the growing demand of seafood products, and at the same time the growing concerns of economic deception involving cases of species misbranding.

For the first time, molecular DNA barcoding has been used to confirm the identity of fish bought in KRI in order to detect potential mislabelling issues (Chapter 4). The usefulness of using DNA barcoding was due to their applicability on identifying species as whole fresh, filleted, frozen or cooked as confirmed by many authors (Filonzi *et al.*, 2010, Cline, 2012, Galal-Khallaf *et al.*, 2014, Nagalakshmi *et al.*, 2016). The COI gene was initially trialled across a range of samples, but due to the poor PCR amplification and some non-specific bands was used only if the sequence was not matched to Cyt b gene. However, it was found that the COI gene was more reliable than Cytb when the sequences were compared to the database. Additionally, all 24 samples sequenced with COI primers were correctly identified. Therefore, using both mitochondrial regions (Cytb and COI) would support and reassure the confirmation of species identity, and both regions has been used previously by other study (Filonzi *et al.*, 2010).

With the Cytb gene, several sequences had high match similarity but were identified as different species, while when sequenced with the COI, it was correctly identified. Although the levels of mislabelling (10%) identified in this study may attribute to a mistake in identification, not intentional mislabelling, this could be a warning that mislabelling may occur anytime and at different stages in the fish supply chain. Because supermarkets tend to protect their brand and

reputation, this could be one of the reasons that mislabelling occurred at street markets and fishmongers, but not in the supermarkets.

Although most of the samples grouped with other samples of the same species in the constructed trees, implying the sequence data and identification were robust, there was no clear grouping based on wild/ farmed common carp. The question remains as to whether farmed fish may be sold as wild for economic profit. Therefore, wild and farmed common carp were not discriminated by this method, but other chemical methods such as fingerprinting profile of composition and NIRS (Chapter 5), or other genetic DNA method such as microsatellite markers could also determine the genetic variations between wild and farmed fish as proposed for further study.

Another limitation of this method is a qualitative approach which can only be used for species detection, but cannot be used to quantify mixed fish species within a sample. An example for this application would be if a fish meal contain more than one fish species, then a PCR-real time could be a quantified detectable technique lower than 0.01 as proposed for further study.

In addition to the main objectives to confirm fish species identity and identifying any potential mislabelling, the sequence data obtained in this study could be uploaded to the online databases of both tools (BLAST and BOLD). This is because when the obtained sequences of local carp were compared to the sequences on databases, no carp species were available from Iraq. Furthermore, samples of *Arabibarbus grypus* were not assigned to any species by the BOLD search engine as there was no COI barcode sequence available for this species at the time of this study.

Therefore, uploading these data would be a great contribution for further research in carp species in KRI and the rest of Iraq where no data were available. It will also contribute to the global scale for research looking at authentication and population of *Arabibarbus grypus.*

Finally, a discrimination experiment was designed to find out if wild and farmed fish could be discriminated. The aim was to evaluate the applicability of using fingerprinting compositional profile and NIRS techniques to discriminate wild from farmed fish (Chapter 5). The importance of discriminating the wild and farmed fish would give consumers the choice of purchasing fish according to their preferred production method. Several consumer researches has been discussed in the literature review regarding the important of discriminating between wild and farmed fish due to their differences on nutritional value, accumulation of minerals and environmental issues (Verbeke and Brunso, 2005, Verbeke *et al.*, 2007, Claret *et al.*, 2014).

First the chemical composition of fish samples were determined (only 22 samples as reference data for NIRS). Because wild and farmed fish may accumulate minerals differently due to their growing environment conditions, a range of minerals (macro and micro minerals) in the muscle fish of wild and farmed common carp were successfully determined using ICP-OES and ICP-MS. This is the first time that minerals have been determined for discrimination of wild from farmed fish in KRI. This study demonstrated that the concentrations of the most detected mineral accumulation in the muscle of wild and farmed fish were slightly different based on their different origin. Although there was not such significant difference between wild and farmed fish, wild fish tend to accumulate higher concentrations of most of the detected minerals. The

concentration of minerals either in wild and farmed fish had the following order: K>P>S>Na>Ca> Mg>Zn>Fe>Cu>Se>Mn>Cr>As>Pb>Co>Cd. Interestingly, the concentrations of heavy trace metals (As, Cd and Pb) on muscle of wild and farmed fish were much lower than permissible limits and in the same species assessed in other countries (Yeganeh *et al.*, 2012). The concentrations of these minerals may reflect the local growing environment, water or feed situations in which these fish were grown or cultivated.

NIR spectroscopy, as a technique based on the unique absorbance profiles at specific wavelengths of the electromagnetic spectrum of the sample component (Shenk *et al.*, 1992), was used in the present study as alternative to the standard chemical methods. Good prediction performance on the values of R^2, RMSECV and RDP were obtained for moisture, protein, fat, ash, and for some macro-minerals (K, P, S, and Mg).

However, for micro-minerals, the prediction model was not satisfactorily accurate. Overall, it was confirmed that NIR spectroscopy is a useful technique for the prediction of macro-mineral contents and chemical composition (moisture, protein, lipids and ash), and that together with the fingerprinting profile, a contribution towards sample groupdiscrimination is possible, but it could not be reliably used for authentication if used alone. However, it has been confirmed in the literature review the applicability of NIR spectroscopy with chemometrics analysis to differentiate between wild and farmed European sea bass (Ottavian *et al.*, 2012). But, a relevant drawback about this technique is it can be used as indirect method, and it requires a reference database. In addition, the detection limit of NIRS is relatively high and other techniques such mathematical chemometrics are required to confirm the results.

Protecting consumers from food fraud and ensuring they have confidence in the food they buy is vital for the highest standards of food safety, quality and traceability. Generally, authenticity issues cover mislabelling claims about food quality, its composition, geographical origin, method of production and undeclared ingredients. The use of qualitative and quantitative tools for studying the consumers' perception has highlighted concerns and issues of food authenticity from animal products.

The highlighted problems were then investigated using different analytical approaches. These are potentially useful to build consumers' confidence and minimise the authenticity issues. Without a full traceability and authenticity system in place, and limited resources for enforcement, consumer's perception should be frequently assessed. There are opportunities for both official bodies and independent organizations to provide tools or schemes to improve confidence; for example, assurance certification that combines auditing and document trail, with product testing to a level that protects from most threats. Major retailers in some countries have explored similar approaches in limited and focused ways.

To conclude, having reliable authentication techniques, such as DNA-based methods, fingerprinting compositional profile and NIRS in place may discourage deliberate replacement in food markets, which in turn may lead to reductions in mislabelling and protecting consumers from fraud. Surely, the enforcement of labelling regulations requires sensitive, reliable, and easy–to-perform analytical methods to verify trace ingredients in processed and unprocessed foods, especially those of animal origin.

Chapter 7

Contribution and limitations of the study, and recommendation for future work

7.1 Contribution of the study

The contribution to methodology and applications in this area of research are highligted below. The consumer study demonstrated that distrust is linked to food authenticity issues. Several key issues with KMP that consumers are most concerned about were identified and linked to several variables.

The novel idea of replacing doner kebab fat content with JA flour was demonstrated, and it was tested in comparison to commercial inulin. It was demonstrated that inulin-rich flour from JA tubers can be extracted with a simple protocol, and that it can be used as alternative to commercial inulin in an area where commercial inulin is unsuitable or not available.

Following field sampling of fish in retail, the study confirmed the applicability of DNA barcoding to verify the species identity, and to identify potential mislabelling for local carp and imported fish from other species. This approach can further followed up by local authorities in KRI to be used for control monitoring for any suspicious cases.

The sequence data obtained in this study especially from local carp is intended to be uploaded to the online data base repositories (BLAST and BOLD). Due to the unavailability of any sequences of local carp from KRI and/ or Iraq in data bases, the contribution is timely. Furthermore, samples of *Arabibarbus grypus* were not assigned to any species by the BOLD search engine as there was no COI barcode sequence available for this species at the time of this study. Therefore, uploading these data would be a valuable contribution for further research in carp species in KRI and the rest of Iraq where no data were

available. It will also contribute to the global scale for researchers looking on authentication and population of *Arabibarbus grypus.*

The usefulness of NIR spectroscopy for predicting compositional and mineral profile of muscle from common carp was demonstrated, but only with partial success with regards to the discrimination of production methods or source.

7.2 Limitations of the study

Inevitably, there are a number of limitations of research which is of exploratory nature, and they could be linked to the protocol design and execution in sampling size, sampling method, data collection and data analysis. These limitations reflect constraints imposed by available resources.

Shortcomings could be linked to the sampling size. The results of the four focus groups only looked at a very small sample of the population of the staff at Plymouth University (20 participants), and the findings of the study may not be characteristic of everyone who has similar perceptions towards KMP.

The limitations with survey are often linked to sampling size and explaining why certain participants have positive attitude towards a particular food products and other a negative image. Qualitative research, such as focus groups used in this study was more appropriate at answering “why” questions.

Regarding sample size of the survey questionnaire, the large standard deviation obtained in this study may affect the reliability of the results. Because a small sample size (241 respondents in the UK and 180 in KRI) affects the reliability of the survey’s results and leads to a higher variability. Although there were

significant differences between most of the demographical characteristics, such as gender, cultural groups, age groups, however, possibly due to differences in their perceptions, there were no significant differences between employment status and educational level, which may be due to small sample size.

Therefore, responses might not necessarily be representative of attitude in the UK and in KRI as a whole, and generalisation of the results should be made with caution thought it is considered that the results are indicative of general attitude of consumers towards authenticity of KMP. However, to have confidence that the survey results are representative, it is important to have large number of participants of around 500 participants within each population with a 95% confidence level and a margin of error less than 5%.

In the experiment of acceptability of replacing fat with JA and commercial inulin, the big challenge was to recruit enough panels to participate on the sensory evaluation. However, only limited volunteer (28 panellists) turned over, and according to BS guideline, it requires 20 or more assessors when considering intensity of the products. In contrast, for considering the degree of preferences which aimed in this study, more than 28 panellists were required.

It was also challenging whether to sequence fish samples using Cytb or COI region in order to confirm species identity and identify potential mislabelling. Therefore several attempts and optimisations were carried out in both regions, and eventually Cytb region was used as premium method. However, COI region was also used for unidentified samples with Cytb and few samples already identified with Cytb in order to reassure the species identify and validate the methods.

Another limitation of this study was to find a tool to discriminating wild and farmed common carp. The differentiation was not possible based on the phylogenetic tree with DNA barcoding (Chapter 4). This limitation was taken further with another experiment as explained below.

The mathematical model system built with NIR spectroscopy for predicting compositional profile of fish samples and discrimination between wild and farmed fish were not satisfactory for some minerals. The big challenge was to build sufficient models for micro-minerals. However, often larger sample sizes may help to build better mathematical modelling system for NIRS prediction. Due to time restriction and limited resources these requirements were not met. Therefore, fingerprinting of compositional and the NIRS data only differentiated wild and farmed samples as a group, but not as a whole on PCA data (Chapter 5).

7.3 Recommendations for future work

The following areas can be studies further:

- Foods can be modified or altered to improve heath quality attributes, becoming functional foods either by adding functional ingredients, or replacing or removing a detrimental component. However, the use of some ingredients and their concentration limits should be carefully considered, to avoid otherwise fraudulent modifications possibly for economic gain. No review papers were found to cover these ingredients that are used for functional purposes, which could possibly be subject to fraudulent practice as they may double up as bulking agents, when consumers are not fully aware of it. A review paper entitled: “food modification: functional attempt or fraud opportunity” is proposed.
- Further research is suggested to include investigating viewpoints of government policy makers, non-government agencies and stakeholders, such as supermarkets or supply chains of animal products, regarding the potential issues of food authenticity. Considering this, further work can be carried out to get a clear view on whether if food authenticity issues could be minimized in the future.
- A better understanding of the physicochemical and functional properties of Jerusalem artichoke tuber is needed due to the changes of inulin characteristics and quantity depending on the maturity and harvesting time. Therefore, further research could determine the best harvesting time and preparation method as a food ingredient to include optimisation of inulin yields, especially in KRI as an alternative source

of inulin compared to commercial inulin. Enzymatic assay can be applied to determine the extracted inulin content to optimise the harvesting time of JA grown in KRI.

- Un-declared gelatine sources in food product labelling have been widely debated for several years among Muslim consumers worldwide. Sources of gelatine should be controlled by suppliers through traceability systems. In addition, various analytical methods have been introduced and developed with certain limitations to differentiate whether gelatine has originated from porcine or bovine sources. Therefore, further research would be needed to ensure food products that contain gelatine are in compliance with halal regulations in KRI. ELISA methods have been proposed as reliable due to their potential to detect species source when raw and when incorporated into processed foods.
- The molecular DNA tool applied in this study was part of a qualitative approach. However, further work like this is needed to quantitatively detect any potential fraud in commercial markets in KRI, especially with products that are susceptible to mixing two or several species such kebab meat products, sausages and fish fingers. Quantitative real-time PCR is the most appropriate method for quantitative detection. Multiplex PCR assay is another option since it allows the detection of multiple species targets in a single assay platform, saving cost and time.
- The toxicity of arsenic is highly dependent on the chemical form of arsenic, especially the inorganic form of arsenic. The separation of

arsenic species and its quantification could be further extended into toxicity studies that could include quantification using a coupled HPLC and ICP-MS instead of the ICP-MS used to determine the concentration of micro-minerals including arsenic in muscle of wild and farmed common carp.

- An evaluation of the present quality control system in KRI needs to find out what measures are in place to protect the food products from fraud, determine the degree of reliance on third party control of certain food standards, and determine whether there are local verifications by sampling at regional entry points and how is that done for local products. Further queries include the actions that the relevant authorities would have in place in case fraud is identified, and who is responsible for such issues and to what extent a company or supermarket can be fined.
- Study the availability of the organizations responsible for certifications of foods in several countries to find out whether this third party could guarantee by their certifications to protect consumers from fraud. Establishing third party organisation for quality assurance system in KRI for certifications could be useful to assess the local and imported food products.

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Appendices

Appendix 2.1: Invitation E-mail draft- Focus group

Hello,

My name is Salih Mustafa Salih, I am a MPhil/ PhD student in the School of Biological Sciences/ Plymouth University. I am looking for volunteers to take part in a focus group sessions to share their opinions as and perception on the kebab meat products. More details are provided in the attached of a briefing sheet.

I would appreciate your help and time to attend a session. If willing to participate, could you please email me at: salih.salih@plymouth.ac.uk, and indicate the most convenient time from below:

	Monday	**Tuesday**	**Friday**
	4th November 2013	5th November 2013	8th November 2013
Morning	11:00 - 12:00	11:00 – 12:00	11:00-12:00
Afternoon	12:00 – 01:00		

Note: Water will be provided during the session

Appendix 2.2: Briefing information sheet- Focus group

Plymouth University **Faculty of Science and Technology, Human Ethics Committee** **Briefing information sheet/ Focus group**
Title of research: Consumers' perceptions of kebab meat products
Name of principal investigator: Salih Mustafa Salih
Aim of research: To investigate the consumers' perception regarding the traceability/ authenticity, quality attributes and species identity of kebab meat products such as doner and shish kebabs
Brief statement of purpose of work: To identify and evaluate the key quality attributes of kebab meat products To identify any issues that consumers are concerned about To obtain and compare the perception of different groups of people
Procedure Participants will be invited by email to attend one session in a group of 6-8 people for about 45-60 min to freely discuss and share their perception, opinions and beliefs about kebab meat products. Audio recording will take place during the session. Please, let us know if you prefer your voice not to be recorded. Participants have the right to withdraw from the session at any time, and withdraw their data. Personal information will not be kept after the study. Risk assessment has been conducted to consider potential risks during this session.
Inclusion/ exclusion criteria: People aged 18 or younger will be excluded
If you are dissatisfied with the way the research is conducted, please contact the principal investigator in the first instance email: salih.salih@plymouth.ac.uk (telephone number 07840612682). If you feel the problem has not been resolved please contact the secretary to the Faculty of Science and technology Human Ethics Committee: Mrs Paula Simson 01752584503. **Thank you for your participation in this session**

Appendix 2.3: Confirmation E-mail- Focus group

Dear ----------

Thank you for your willingness to participate in a focus group session as follows:

Date: e.g., Monday 4th November 2013**, Time:** 11:00-12:00 am

Place: Portland Square Building (PSQ) / 4th Floor - Seminar room A415

If you need further directions to attend the session, or will not be able to attend for any reason please call (07840612682).

Looking forward to see you

Appendix 2.4: Consent information sheet and consent form- Focus group

Plymouth University

Faculty of Science and Technology, Human Ethics Committee

Consent information and consent form/ Focus group

Title of research: Consumers' perceptions of kebab meat products

Name of principal investigator: Salih Mustafa Salih

Consent information:

The objectives of this research have been explained to me:

- I know that I am free to withdraw from this session at any time, and I have right to withdraw my data.
- I know that personal information will not be kept after the study.
- Audio recording will take place during the session. If you prefer you voice not to be recorded, please inform us.
- I am aware that risk assessment has been conducted considering potential risks during this session.
- I have read the information sheet and considered the consent information.
- Under these circumstances, I agree to participate in the session.

Consent form, signatures/ comments - Focus group

No.	Name of participants	Signatures	Comments
1			
2			
3			
4			
↓			
20			

Appendix 2.5: List of questions asked during the focus group discussions

1- Are you familiar with kebab meat products such as shish and doner and to what extent are you satisfied, preferred, trusted and eat more than other regardless the price?

2- Why do you eat kebab meat products in comparison to other fast foods?

3- What influence your decision to purchase kebab products?

4- What are the main qualities attributes that you like about the kebab meat products?

5- How aware are you about the types of meat species, and the levels of meat content in a kebab meal?

6- Regardless the meat content and, what others ingredients should be in a kebab meal and what should not be in?

7- What do you think about the idea of adding dietary fibre into kebab meat products?

8- Have you ever thought about kebab labelling, especially in the catering services?

9- How do you think about nutritional value of kebabs?

10- Are there any safety or hygiene issues that you would be concerned about?

Appendix 2.6: Focus group guideline

Guideline used to conducting the focus group
Introduction to the focus group (5 min) The introduction was started by welcome words; introduce moderator, co-moderator and an overview of the topic, purposes of the focus group and the objectives. Ground rules are explained by moderator and then everyone had a short self-introduction.
Welcome word and Self-introduction Good morning/ afternoon and welcome to our focus group session. Thanks for taking the time to join us to talk about consumer perception of kebab meat products. My name is Salih, I am a M.Phil./ PhD student in the School of Biological Science/ Plymouth University. I will be the moderator for running this session and assisting me is Calvin will take notes and engage with recording stuff as we are tape recording the session because we don't want to miss any of your helpful comments. The purpose of today session is to investigate your perception, opinion and beliefs on kebab meat products such as doner and shish kebab. An approval ethical certificate is obtained in order to run this session that indicate your right to withdraw and maintains data confidentiality. Some pictures of kebab products are provided to stimulate the discussion during the session. Water is provided for creating a comfortable and relaxed atmosphere.
Ground rules during the session • Let is all turn off our mobile phones so we are not interrupted. • Everyone should have a chance to talk in turn, so that all questions are discussed and everyone's ideas are heard. • Please feel free to share your point of view even if it differs from others. • Please do not interrupt when someone else is talking.
Participants-self-introduction Before we start, we have placed name cards on the table on front of you to help us remember each other's names. So, may I ask everyone to have a short self-introduction, e.g. your name, where are you from and other information you may be interested in and your favourite fast food?
During the discussion (30-45 min) A list of questions was prepared for the discussion structured around the perception and concerns of kebab consumption.
Ending the session (2-3 min) This is all for the session, do you have any other opinion, or if there anything else in mind or any missing question in relation to the topic that you would like to share? Thank you for your participation. What you have shared with us is appreciated and will be kept confidentially. A small presents were given to each participant at the end of each session.

Appendix 2.7: Invitation E-mail daft and information sheet- WBS in the UK

Hello,

My name is Salih Mustafa Salih, I am a M.Phil./ PhD student in the School of Biological Sciences/ Plymouth University, carrying out a questionnaire survey investigating the consumer perception of the attributes of kebab meat products such as doner and shish kebab. I would be very grateful if you could spare about 10 minutes to complete this questionnaire.

Note: Participants must be over 18 years.

Confidentiality:

Information gathered will only be used for this study and will not be kept. The questionnaire is anonymous, and that date will not be linked to individuals.

Right to withdraw: You can withdraw from the questionnaire at any time. Due to anonymity, data cannot be withdrawn once submitted.

If you are dissatisfied with the way the research is conducted, please contact the principal investigator in the first instance: e-mail salih.salih@plymouth.ac.uk. If you feel the problem has not been resolved please contact the secretary to the Faculty of Science and technology Human Ethics Committee: Mrs Paula Simson 01752584503.

If you want to keep a copy of this page, please click here (Download Info Sheet)

To proceed with the survey, click on this link:

Web link

Faithfully Yours,

Salih Mustafa Salih

Appendix 2.8: Invitation E-mail daft and consent form with information sheet- WBS in KRI

ڕاپرسی دەربارەی بەرهەمەکانی کەباب/ گەس لە هەرێمی کوردستان

سڵاو هاوڕێیان:

من ناوم صالح مصطفی صالح، قوتابی دکتۆرام لە زانکۆی پلیموث لە وواڵاتی بەریتانیا. هەڵدەستم بەئەنجامدانی ڕاپرسیەک دەربارەی ڕاو بۆچوونی بەکارهێنەر (خەڵک) لە سەر بەرهەمەکانی گوشت (کەباب و گەس/ سەندەویچ).

زۆر سوپاسی هاوکاریتان دەکەم ئەگەر بتوانی ١٠ خولێک لە کاتەکەت تەرخان کەی بۆ تەواو کردنی ئەم ڕاپرسیە بە وەڵام دانەوەی گشت پرسیارەکان.

تێبینی: بەشداربوو دەبێت لە ١٨ ساڵ کەمتر نابێت.

- زانیاری کوکراوە تەنها بۆ ئەم ڕاپرسیە بەکار دێت وە هەڵناگیرێت دواتر

- ئەم ڕاپرسیە بێ ناو نیشانە (ناوەکان نانووسرێت)

مافی کشانەوە لە ڕاپرسیەکە:

ئەتوانیت لەم ڕاپرسیە پاشەکشێ بیەوە لە هەر کاتێک دا، لە بەر ئەوەی ئەم ڕاپرسیە بی ناو نیشانە، ناتوانیت پاشەکشێ بیکەی پاش ئەوەی تەواوت کردو ناردێت.

ئەگەر ڕات لە سەر ئەم ڕاپرسیە نیە، تکایە پەیوەندی بکە بە توێژەری سەرەکەی بە ڕێگای ئیمیل:
salih.salih@plymouth.ac.uk

ئەگە هەر جاراسەر نە بێت، ئەتوانیت بەیوەندی بکەی بە (Mrs Paula Simson 01752584503)

سکرتێری فاکولتی زانست و تکنلوجیا/ لیژنەی ڕەوشتی مروڤ/ زانکۆی پلیموث.

ئەگەر دەتەوێت کۆپیەک لەم زانیاریانە، تکایە کلیک لە سەر لێرە بێکە **(داونلۆدی فورمی زانیاری)**.

بۆ دەست پێ کردنی ڕاپرسیەکە، کلیک لە سەر ئەم لینکە بکە:

ویپ لینک (**Web link**)

Appendix 2.9: English WBS questionnaire

Kebab meat products

*** 1. What would you consider to be your cultural background?**

- British/ European
- Middle Eastern- Kurdish
- Middle Eastern- Other
- Other Cultural Background

*** 2. How long have you been in the UK?**

- Less than 6 Months
- 6 Months to 11 Months
- 1 to 3 Years
- 3 to 5 Years
- More than 5 years

Other (please specify)

*** 3. What is your Gender?**

- Female
- Male

*** 4. What is your Age?**

*** 5. Education Level:**

- High school or below
- College/University
- Graduated

*** 6. Are you?**

- Student
- Employed
- Unemployed
- Retired

Other (please specify)

*** 7. Have you ever tried the following products (Definitions provided below):**

	Yes	No
Doner kebab/ Shawarma: A meat dish usually from sliced or minced lamb, beef and chicken meat marinated and slowly roasted on rotating spit served on bread with condiments.		
Beef burger: A sandwich of cooked patty usually from minced beef meat		
Shish kebab: A meat dish consisting of pieces or minced lamb, beef or chicken roasted on skewers and commonly served on bread with condiments.		

*** 8. How often do you eat a kebab meal?**

- ◯ Twice or more a week
- ◯ Once a week
- ◯ Twice a month
- ◯ Special occasions
- ◯ Rarely

*** 9. How much do you usually spend on eating a kebab meal?**

- ◯ Less than £ 5/ Month
- ◯ £5-10/ Month
- ◯ £10-20/ Month
- ◯ £20-30/ Month
- ◯ £30-40/ Month
- ◯ £40-50 or more/ Month

*** 10. How much would you trust the following products?**

	No trust at all	Some distrust	Neutral	Some trust	Full trust
Pasty	◯	◯	◯	◯	◯
Fish and chips	◯	◯	◯	◯	◯
Doner kebab	◯	◯	◯	◯	◯
Sausages	◯	◯	◯	◯	◯
Pizza	◯	◯	◯	◯	◯
Vegetable burger	◯	◯	◯	◯	◯
Shish kebab	◯	◯	◯	◯	◯
Beef burger	◯	◯	◯	◯	◯

*** 11. For each of the following attributes, rate its importance when you choose a kebab meal.**

	Not important	Little importance	Somehow important	Most important
Aroma	◯	◯	◯	◯
Convenience	◯	◯	◯	◯
Wholesomeness	◯	◯	◯	◯
Following friends/ family	◯	◯	◯	◯
Price	◯	◯	◯	◯
Flavour	◯	◯	◯	◯
Safety of meal	◯	◯	◯	◯
Special occasions	◯	◯	◯	◯
Taste	◯	◯	◯	◯
Trying something different	◯	◯	◯	◯
Tenderness	◯	◯	◯	◯
Past experience	◯	◯	◯	◯
Fashion	◯	◯	◯	◯
Overall appearance	◯	◯	◯	◯
Nutritional value	◯	◯	◯	◯
Composition of meal	◯	◯	◯	◯
Authentic/ Original	◯	◯	◯	◯
Halal / kosher	◯	◯	◯	◯
Freshness/ freshly made	◯	◯	◯	◯

Next

Kebab meat products

*** 12. How much would be acceptable for you to be the meat content in a doner kebab meal?**

- Less than 25%
- 30%
- 35%
- 40%
- 45%
- 50%
- 55%
- 60%
- 65%
- 70%
- 75%
- 80%
- 85%
- 90%
- 95%
- 100%

13. How much would be acceptable for you to be the meat content in a shish kebab meal?

- Less than 25%
- 30%
- 35%
- 40%
- 45%
- 50%
- 55%
- 60%
- 65%
- 70%
- 75%
- 80%
- 85%
- 90%
- 95%
- 100%

*** 14. How much do you think is the actual meat content in a doner kebab meal?**

- Less than 25%
- 30%
- 35%
- 40%
- 45%
- 50%
- 55%
- 60%
- 65%
- 70%
- 75%
- 80%
- 85%
- 90%
- 95%
- 100%

*** 15. How much do you think is the actual meat content in a shish kebab meal?**

- Less than 25%
- 30%
- 35%
- 40%
- 45%
- 50%
- 55%
- 60%
- 65%
- 70%
- 75%
- 80%
- 85%
- 90%
- 95%
- 100%

*** 16. If you consider the meat content of kebab meal to be less than 100%, what do you think about other main ingredients?**

	Shall not be used	Acceptable in low proportion	Acceptable in medium Proportion	Acceptable in any proportion
Wheat (flour)				
Fat				
Starch				
Mechanically recovered meat (MRM)				
Soybean ingredient				
Bread				
Alternative meat cuts				
Wheat bulgur (cracked wheat)				
Dietary fibre (e.g. inulin)				

*** 17. If these meats are to be part of a kebab meal, please indicate your preference meats and which you don't expect.**

	Unexpected meat	Preferred meat
Mutton (meat from sheep of age over 12 months old)	◯	◯
Horse/ Donkey	◯	◯
Chicken	◯	◯
Fish	◯	◯
Pork	◯	◯
Lamb (meat from sheep of age under 12 months old)	◯	◯
Goat/ Kid	◯	◯
Beef	◯	◯

*** 18. How would you feel if your kebab meal contains the following:**

	Strongly disappointed	Very disappointed	Slightly disappointed	Don't mind
Undeclared meat species	◯	◯	◯	◯
Undeclared non-meat protein soy protein, gelatine, gluten	◯	◯	◯	◯
Undeclared horse meat	◯	◯	◯	◯
High content of fat and salt	◯	◯	◯	◯
Undeclared vegetable	◯	◯	◯	◯
Adding flour, starch and bread	◯	◯	◯	◯
Undeclared non-halal meat	◯	◯	◯	◯
Undeclared mechanically recovered meat (MRM)	◯	◯	◯	◯

*** 19. To what extent are you concerned about the following attributes when you choose a kebab meal?**

	Not concerned	Slightly concerned	Somehow concerned	Very concerned
Horse meat presence	◯	◯	◯	◯
Amount of fat and salt	◯	◯	◯	◯
Calories on meal	◯	◯	◯	◯
Price	◯	◯	◯	◯
Genetically Modified Ingredients (GM)	◯	◯	◯	◯
Ingredients/ Composition of meal	◯	◯	◯	◯
Hygienic conditions	◯	◯	◯	◯
Type of meat species	◯	◯	◯	◯

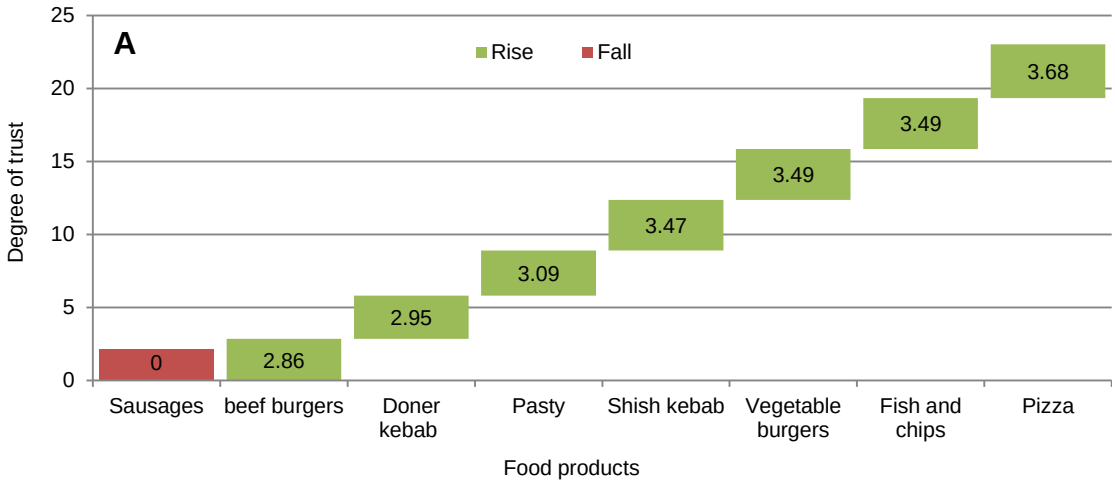
*** 20. In regards to kebab meals, how much do you agree or disagree with the following statements?**

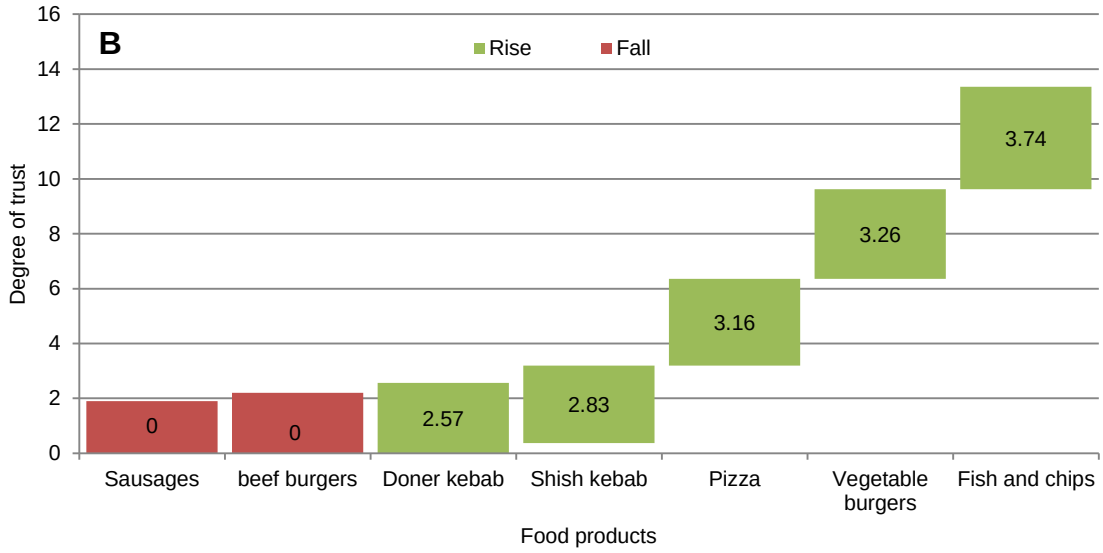
	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
If kebab meal contains (soy protein or gluten) could produce potential allergies and therefore, it must be declared	○	○	○	○	○
If the meat content was under 65%, I would still eat kebab meal	○	○	○	○	○
I never worry about the quality of my kebab meal	○	○	○	○	○
Information about kebab products influence whether or not I buy kebab products	○	○	○	○	○
I do not trust any meat that is minced regardless where I buy it	○	○	○	○	○
I don't think kebab products are healthy	○	○	○	○	○
Having labelling on kebab will not affect my decisions when buying kebab products	○	○	○	○	○
I am always very satisfied with kebab products	○	○	○	○	○
I would prefer kebab products to be labelled and with all ingredients	○	○	○	○	○
Knowing the origin of the meat will not influence my choice to eat kebab meal	○	○	○	○	○
I will pay more if the kebab products are well labelled	○	○	○	○	○
I would like to avoid eating kebab products	○	○	○	○	○
Due to recent incident with horse meats, I eat less processed meat products including doner kebab	○	○	○	○	○

Prev Done

Thank you very much for completing this questionnaire

Appendix 2.10: Waterfall charts of ranking the degree of trust in certain fast food products, A (UK , n=241), B (KRI, n=180)





Appendix 2.11: Association between socio-demographic characteristic (independent variables) and dependent variables (results of cross-tabulation and Chi square test using SPSS/ full data of all results were not shown)

Association between independent and dependents variables in the UK (n=241).	**Asymp.sig (2-sided)**	**Difference**
Q7- Have you ever tried shish kebab? vs Cultural Groups	0.004	Significant
Have you ever tried beef burger? vs Cultural Groups	0.001	Significant
Have you ever tried shish kebab? vs Period in the UK	0.728	Not significant
Have you ever tried beef burger? vs Period in the UK	0.008	Significant
Have you ever tried doner kebab? vs Gender	0.785	Not significant
Have you ever tried beef burger? vs Gender	0.004	Significant
Q8- Frequency of eating kebabs vs Cultural Groups	0.0001	Highly significant
Frequency of eating kebabs vs Period lived in the UK	0.009	Significant
Frequency of eating kebabs vs Gender	0.020	Significant
Frequency of eating kebabs vs Age Groups	0.015	Significant
Q9- Spending money on kebabs vs Cultural Groups	0.000	Highly significant
Spending money on kebabs vs Age Groups	0.0001	Highly significant
Spending money on kebabs vs Gender	0.004	Significant
Spending money on kebabs vs Education level	0.609	Not Significant
Q10- Trust on pizza vs Cultural Groups	0.012	Significant
Trust on beef burger vs Cultural Groups	0.034	Significant
Trust on fish and chips vs Cultural Groups	0.001	Significant
Trust on pasty vs Cultural Groups	0.005	Significant
Trust on doner kebab vs Cultural Groups	0.022	Significant
Trust on sausages vs Cultural Groups	0.0001	Highly significant
Trust on shish vs Gender	0.021	Significant
Trust on shish vs age groups	0.046	Significant
Trust on beef-burger vs age groups	0.067	Not Significant
Trust on veg burger vs age groups	0.036	Significant
Trust on sausages vs age groups	0.012	Significant
Trust on sausages vs Period lived in the UK	0.003	Significant
Trust on sausages vs Employee status	.045	Significant
Q12 and14- Acceptable meat content on shish kebab vs Actual meat content on shish kebab	0.0001	Highly significant
Q13 and 15- Acceptable meat content on doner kebab vs Actual meat content on doner kebab	0.0001	Highly significant

Appendix 2.11 Continues

Association between independent and dependents variables in KRI (n=180)	**Asymp.sig (2-sided)**	**Difference**
Have you ever tried shish kebab? * Gender	0.029	Significant
Have you ever tried doner kebab? * Gender	0.200	Not Significant
Have you ever tried beef burger? * Gender	0.450	Not Significant
Have you ever tried shish kebab? Vs age	0.288	Not Significant
Have you ever tried doner kebab? Vs age	0.002	Significant
Have you ever tried beef-burger? Vs employee	0.016	Significant
Frequency of eating kebab vs Gender	0.038	Significant
Frequency of eating kebab vs Age groups	0.183	Not Significant
Frequency of eating kebabs vs Education levels	0.063	Not Significant
Frequency of eating kebab vs Employee status	0.803	Not Significant
spending money on kebab vs Gender	0.398	Not Significant
spending money on kebab vs Age Groups	0.097	Not Significant
spending money on kebab vs Education levels	0.018	Significant
spending money on kebab vs Employee status	0.200	Not Significant
Trust on pizza vs Gender	0.044	Significant
Trust on beef burger vs Gender	0.129	Not Significant
Trust on shish vs Gender	0.086	Not Significant
Trust on fish and chips vs Gender	0.263	Not Significant
Trust on doner vs Gender	0.534	Not Significant
Trust on sausages vs Gender	0.771	Not Significant
Trust on veg burger vs Gender	0.418	Not Significant
Trust on fish and chips vs Education levels	0.029	Significant
Trust on sausages vs Education levels	.639	Not Significant
Q10,12- Acceptable meat content on doner kebab vs Actual meat content on doner kebab/ Kurdistan	0.007	Significant
Q11,13- Acceptable meat content on shish kebab vs Actual meat content on shish kebab/ Kurdistan	0.0001	Highly significant

Appendix 2.11 continue

1- Does cultural groups affect the frequently of eating KMP in the UK?

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Frequency of eating kebabs * Cultural Groups	241	100.0%	0	0.0%	241	100.0%

Q8 Frequency of eating kebabs * Q1 Cultural groups Cross-tabulation

			Q1 Cultural groups				Total
			British/ European	Kurdish	Middle Eastern	Other	
Q8 Frequency of eating kebabs	Twice or more a week	Count	0	7	4	0	11
		% within Q8 Frequency of eating kebabs	0.0%	63.6%	36.4%	0.0%	100%
	Once a week	Count	3	12	19	3	37
		% within Q8 Frequency of eating kebabs	8.1%	32.4%	51.4%	8.1%	100%
	Twice a month	Count	6	15	19	6	46
		% within Q8 Frequency of eating kebabs	13.0%	32.6%	41.3%	13.0%	100%
	Special occasions	Count	10	35	31	6	82
		% within Q8 Frequency of eating kebabs	12.2%	42.7%	37.8%	7.3%	100%
	Rarely	Count	27	6	17	15	65
		% within Q8 Frequency of eating kebabs	41.5%	9.2%	26.2%	23.1%	100%
Total		Count	46	75	90	30	241
		% within Q8 Frequency of eating kebabs	19.1%	31.1%	37.3%	12.4%	100%

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	55.743[a]	12	.000
Likelihood Ratio	57.764	12	.000
Linear-by-Linear Association	1.849	1	.174
N of Valid Cases	241		

a. 5 cells (25.0%) have expected count less than 5. The minimum expected count is 1.37.

2- Does gender affect the frequently of eating KMP in the UK?

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Frequency of eating kebabs * Gender	241	100.0%	0	0.0%	241	100.0%

Q8 Frequency of eating kebabs * Q3 Gender Cross-tabulation

			Q3 Gender		Total
			Male	Female	
Q8 Frequency of eating kebabs	Twice or more a week	Count	8	3	11
		% within Q8 Frequency of eating kebabs	72.7%	27.3%	100%
	Once a week	Count	31	6	37
		% within Q8 Frequency of eating kebabs	83.8%	16.2%	100%
	Twice a month	Count	33	13	46
		% within Q8 Frequency of eating kebabs	71.7%	28.3%	100%
	Special occasions	Count	60	22	82
		% within Q8 Frequency of eating kebabs	73.2%	26.8%	100%
	Rarely	Count	35	30	65
		% within Q8 Frequency of eating kebabs	53.8%	46.2%	100%
Total		Count	167	74	241
		% within Q8 Frequency of eating kebabs	69.3%	30.7%	100%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	11.711[a]	4	.020
Likelihood Ratio	11.694	4	.020
Linear-by-Linear Association	7.612	1	.006
N of Valid Cases	241		

a. 1 cells (10.0%) have expected count less than 5. The minimum expected count is 3.38.

3- Does gender affect the frequently of eating KMP in KRI?

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Frequency of eating kebab * Gender	180	100.0%	0	0.0%	180	100.0%

Q6 Frequency of eating kebabs * Q1 Gender Cross-tabulation

			Q1 Gender		Total
			male	female	
Q6 Frequency of eating kebabs	Twice or more a week	Count	28	11	39
		% within Q6 Frequency of eating kebabs	71.8%	28.2%	100.0%
	Once a week	Count	29	5	34
		% within Q6 Frequency of eating kebabs	85.3%	14.7%	100.0%
	Twice a month	Count	34	10	44
		% within Q6 Frequency of eating kebabs	77.3%	22.7%	100.0%
	Special occasions	Count	13	11	24
		% within Q6 Frequency of eating kebabs	54.2%	45.8%	100.0%
	Rarely	Count	23	16	39
		% within Q6 Frequency of eating kebabs	59.0%	41.0%	100.0%
Total		Count	127	53	180
		% within Q6 Frequency of eating kebabs	70.6%	29.4%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	10.160[a]	4	.038
Likelihood Ratio	10.323	4	.035
Linear-by-Linear Association	4.650	1	.031
N of Valid Cases	180		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 7.07.

4- Does age groups affect the frequently of eating KMP in the UK?

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Q8 Frequency of eating kebab * Age Groups	241	100.0%	0	0.0%	241	100.0%

Q8 Frequency of eating kebabs * Q4 Age groups Cross-tabulation

			Q4 Age groups					Total
			19-25	26-30	31-35	36-40	over 40	
Q8 Frequency of eating kebabs	Twice or more a week	Count	4	4	3	0	0	11
		% within Q8 Frequency of eating kebabs	36.4%	36.4%	27.3%	0.0%	0.0%	100%
	Once a week	Count	3	14	12	4	4	37
		% within Q8 Frequency of eating kebabs	8.1%	37.8%	32.4%	10.8 %	10.8%	100%
	Twice a month	Count	4	19	16	5	2	46
		% within Q8 Frequency of eating kebabs	8.7%	41.3%	34.8%	10.9 %	4.3%	100%
	Special occasions	Count	7	29	30	10	6	82
		% within Q8 Frequency of eating kebabs	8.5%	35.4%	36.6%	12.2 %	7.3%	100%
	Rarely	Count	19	20	14	2	10	65
		% within Q8 Frequency of eating kebabs	29.2%	30.8%	21.5%	3.1%	15.4%	100%
Total		Count	37	86	75	21	22	241
		% within Q8 Frequency of eating kebabs	15.4%	35.7%	31.1%	8.7%	9.1%	100%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	30.520[a]	16	.015
Likelihood Ratio	31.500	16	.012
Linear-by-Linear Association	.001	1	.973
N of Valid Cases	241		

a. 9 cells (36.0%) have expected count less than 5. The minimum expected count is .96.

5- Does the cultural groups in the UK affect the respondents' trust on sausages?

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Q1 Cultural Groups * Q10 Trust on Sausages	241	100.0%	0	0.0%	241	100.0%

Q1 Cultural Groups * Q10 Trust on Sausages Cross-tabulation

			Q10 Trust on Sausages					Total
			No trust at all	some distrust	Neutral	Some trust	Full trust	
Q1 Cultural Groups	British/ European	Count	4	16	5	17	4	46
		% within Q1 Cultural Groups	8.7%	34.8%	10.9%	37.0%	8.7%	100%
	Kurdish	Count	33	17	19	6	0	75
		% within Q1 Cultural Groups	44.0%	22.7%	25.3%	8.0%	0.0%	100%
	Middle Eastern	Count	55	13	17	4	1	90
		% within Q1 Cultural Groups	61.1%	14.4%	18.9%	4.4%	1.1%	100%
	Other cultural background	Count	6	7	8	8	1	30
		% within Q1 Cultural Groups	20.0%	23.3%	26.7%	26.7%	3.3%	100%
Total		Count	98	53	49	35	6	241
		% within Q1 Cultural Groups	40.7%	22.0%	20.3%	14.5%	2.5%	100%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	70.831[a]	12	.000
Likelihood Ratio	72.515	12	.000
Linear-by-Linear Association	8.099	1	.004
N of Valid Cases	241		

a. 5 cells (25.0%) have expected count less than 5. The minimum expected count is .75.

Appendix 3.1: List of ingredients of the three commercial doner kebab samples (references) obtained from local kebab shops in Plymouth, UK

Sample ID	Supplier	Meat content	Other ingredients
Kebab shop1	Olympic All Lamb	Lamb 83% Lamb fat	Water, soya protein, yogurt (whole and skimmed milk preservative: E202), rusks (wheat gluten, yeast), textured soya protein, salt, modified maize starch, soya fibre, onion, mixed spices and herbs, dextrose, stabiliser: E450, flavour enhancer: E621
Kebab shop2	Cassius halal kebab	Lamb 69%, Lamb fat, beef 6%	Water, rusks (wheat), onion, yogurt, salt, isolate (Soya), textured vegetable protein (soya), spices, flavour enhancer: E621, dextrose, emulsifier E451, preservative E223, sulphites May contain traces of mustard
Kebab shop3	Veli's doner kebab manufacturer	Lamb 85%, Lamb fat	Water, rusks (wheat, gluten), textured soya flavour, salt, 1.5%protein, wheat flour, flavour enhancer: E621, onion, dextrose, spices, stabilizer, E451, E452, E452, allergens including cereals containing gluten

Appendix 3.2: Invitation Email draft- Sensory evaluation

Hello,

My name is Salih Mustafa Salih, I am a MPhil/ PhD student in the School of Biological Sciences/ Plymouth University. I am looking for volunteers to participate in my study; the sensory evaluation of doner kebab.

The exercise will take place as ONE session where the eating (sensory) qualities of 5 types of doner kebab will be evaluated individually by a panel of volunteers.

The whole exercise will take approximately 10-15 minutes of your time including briefing, and assessment of samples with the aid of a questionnaire.

More details are provided in the attached form of a briefing sheet. If willing to participate, could you please email me at: salih.salih@plymouth.ac.uk

I would very much appreciate your help if you could spare some time please come to the **Food and Nutrition Laboratory, Ground Floor Link Building on Friday 11th April either from 10:30 to 12.30 or between 02:30 to 15.30.**

Appendix 3.3: Briefing sheet and consent form- Sensory evaluation

Plymouth University **Faculty of Science and Technology** **Briefing sheet/ Sensory evaluation**
Title of research: Sensory evaluation of doner kebab
Name of principal investigator: Salih Mustafa Salih
Ingredients: The samples that will be consumed by participants contain: lamb meat, lamb fat, water, dietary fibre (inulin), spices and salt.
Allergy advice: No allergens identified You will be served some samples of Doner kebab supplemented with different levels of dietary fibre (inulin) and you are asked to evaluate, and then taste one sample at the time and give score to each product by ticking **(√)** in the correct box for each property. Please feel free to leave your name and any comments on the space provided or on the reverse of the sheet.
Consent information: The objectives of this research have been explained to me: • I know that I am free to withdraw from this panel at any time, and I have right to withdraw my data. • I know that personal information will not be kept after the exercise. • I am aware that risk assessment was conducted to preclude potential risks during this work. • Under these circumstances, I agree to participate in the panel.
If you are dissatisfied with the way the research is conducted, please contact the principal investigator in the first instance email: salih.salih@plymouth.ac.uk (telephone number 07840612682). If you feel the problem has not been resolved please contact the secretary to the Faculty of Science and technology Human Ethics Committee: Mrs Paula Simson 01752584503. **Thank you for your participation in this panel**

Appendix 3.4: Attributes explanation- Sensory evaluation

Overall appearance: How much do you like/ dislike the product looks?

Flavour: How much do you like/ dislike the smell and the taste of the product while eating?

Colour: How much do you like/ dislike the colour of the product?

Texture: How much do you like/ dislike the consistency and cohesiveness of the product in the mouth and how it holds together when beginning to chew?

Chewiness: The amount and nature of chewing required.

Juiciness: How much do you like/ dislike the juiciness of the product while eating?

Fattiness: How much do you like/ dislike the oiliness/ greasiness of the product?

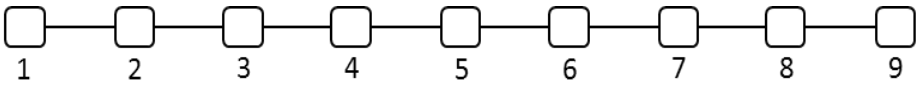
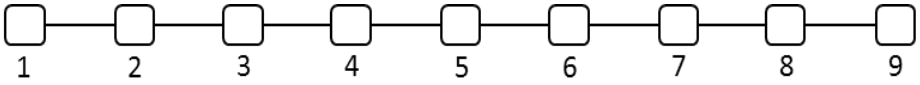
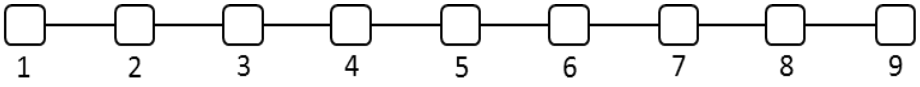
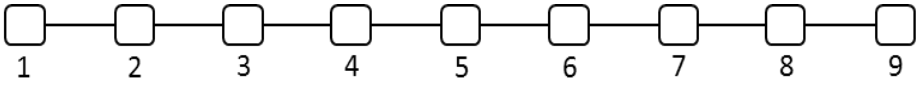
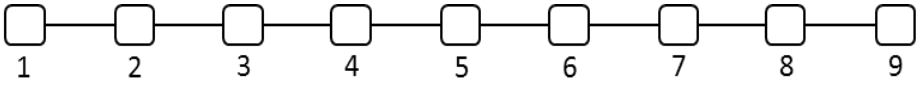
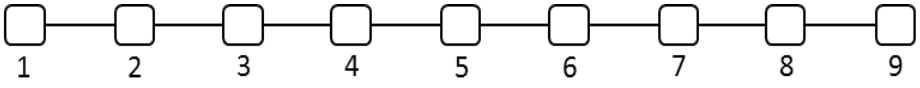
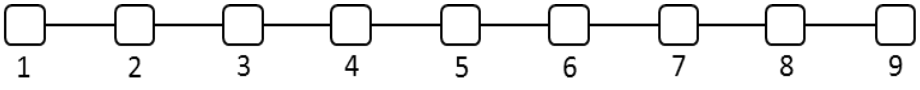
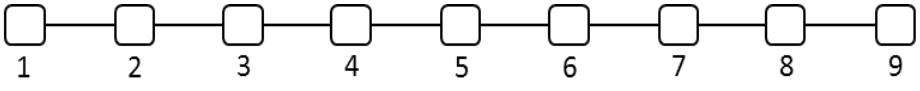
Overall acceptability: To what extent do you like/ dislike the product in general?

Appendix 3.5: Sensory evaluation form of doner kebab with addition of inulin using nine point hedonic scales

Panellists code: …….. Sample code: ……..

Please evaluate and indicate your opinion about each attribute by marking (X) in a suitable box for each attribute. Descriptions of the attributes are provided in a separate page.

Please make sure that your results are placed under the correct code.

Sensory attributes	1 dislike extremely	5 neither like or dislike	9 Like extremely
Overall appearance			
Flavour			
Colour			
Texture			
Chewiness			
Juiciness			
Fattiness/ greasiness			
Overall acceptability			

Additional comment (if any): ……...

……

Appendix 3.6: Calculation of inulin in the tubers of JA and meat products

The amount of fructan presented in the sample and expressed as inulin was calculated according to the following equations:

The absorbances of all samples were determined by calculating the differences between (**A2**-**A1)** for sugars "**S**" and fructan + sugars "**F+S**". The values for **ΔA** $_S$ and **ΔA** $_{F+S}$ were described below.

Determination of D-fructose + D-glucose in the "**S**" sample: **ΔA** $_S$ = (A2-A1)

Determination of D-fructose + D-glucose in the "**F+S**" sample: **ΔA** $_{F+S}$ = (A2-A1)

The concentration of "**S**" and "**F+S**" was calculated as follows:

$$C = \frac{V \times MW}{\varepsilon \times d \times v} \times \frac{0.9}{0.2} \times \Delta A \qquad [g/L]$$

Where:

V = final volume [mL]

MW = molecular weight of D-glucose or D-fructose [g/mol]

Ɛ = extinction coefficient of NADPH at 340 nm = 6300 [l x mol-1 x cm-1]

d = light path [cm]

v = sample volume [mL]

0.9/0.2 = 0.2 mL of sample was incubated with 0.2 mL sucrase / maltase enzyme and 0.5 mL acetate buffer added (total 0.9 mL); 0.2 mL of this was taken for incubation with fructanase enzymes (i.e. 0.2 mL removed from 0.9 mL).

The equation for ''**S**'' as follows:

$$C = \frac{2.62 \times 180.16}{6300 \times 1 \times 0.2} \times \frac{0.9}{0.2} \times \Delta A_S \qquad [g/L]$$

C = 1.6858 × **ΔA** $_S$ ……..…….…….. [g/L]

The equation for "**F+S**" as follows:

$$C = \frac{2.62 \times 180.16}{6300 \times 1 \times 0.2} \times \frac{0.9}{0.2} \times \quad [g/L]$$

$C = 1.6858 \times \mathbf{\Delta A}_{F+S}$ ..... [g/L]

For ''fructan'':

$C_{(fructan)} = C_{(F+S)} - C_{(S)}$ .....[g/L]

Content of fructan as g/100g was calculated as follows:

$$\text{Fructan} = \frac{C_{\text{Fructan}}\ [\text{g/L sample}}{\text{Weight}_{\text{sample}}\ [\text{g/L sample solution}]} \times 100 \times \frac{162}{180} \quad [g/L]$$

Where:

162/180 = factor to convert from free fructose and glucose as determined, to anhydrofructose and anhydroglucose as occurs in fructan.

Where 1.0 g sample is extracted in 500ml: "Weight $_{sample}$ [g/L sample solution]" = 2

(For sample containing 12-100% fructan such JA).

Where 1.0 g sample is extracted in 50ml: "Weight $_{sample}$ [g/L sample solution]" = 20

(For sample containing 0-12% fructan such as meat products).

Where 200 mg sample is extracted in 100ml: "Weight $_{sample}$ [g/L sample solution]" = 20

(For sample of fructan control flour).

Appendix 4.1: List of all collected fish samples and labelling information

Sample code	Fish sold as (local name)	Processing state	Sample location	Sample size	Origin	Price GBP£/kg	Mislabelling?
FCC	Farmed common carp	Cooked	Restaurants	8	Local	£8-10	No
FCC	Farmed common carp	Fresh whole	Direct from farm	20	Local	£4-5	No
FCC	Farmed common carp	Fresh whole	Fishmongers	7	Local	£4-5	No
WCC	Wild common carp	Fresh whole	Street markets	8	Local	£6-7	Yes (all samples)
WCC	Wild common carp	Fresh whole	Fishmongers	23	Local	£6-7	Yes (4 out of 23)
WUS	Wild unknown species	Fresh whole	Fishmongers	4	Local	£3-4	N/A
WUS	Wild unknown species	Fresh whole	Street markets	8	Local	£3-4	N/A
SH	Shabbout	Fresh whole	Fishmongers	9	Local	£8-10	No
SB	Sea bass	Fresh whole	Supermarkets	4	Import	£11-12	No
SA	Salmon	Filleted frozen	Supermarkets	4	Import	£13-14	No
SA	Salmon	Fresh filleted	Supermarkets	4	Import	£16-17	No
SR	Sardine	Fresh whole	Supermarkets	4	Import	£10-11	No
SI	Sultan Ibrahim	Fresh whole	Supermarkets	4	Import	£12-14	No
KF	Kingfish	Filleted fresh	Supermarkets	3	Import	£16-18	No
MK	Mackerel	Fresh whole	Supermarkets	3	Import	£12-14	No
NP	Nile Perch	Filleted fresh	Supermarkets	3	Import	£14-16	No
CF	Cuttlefish	Filleted fresh	Supermarkets	4	Import	12-14	No

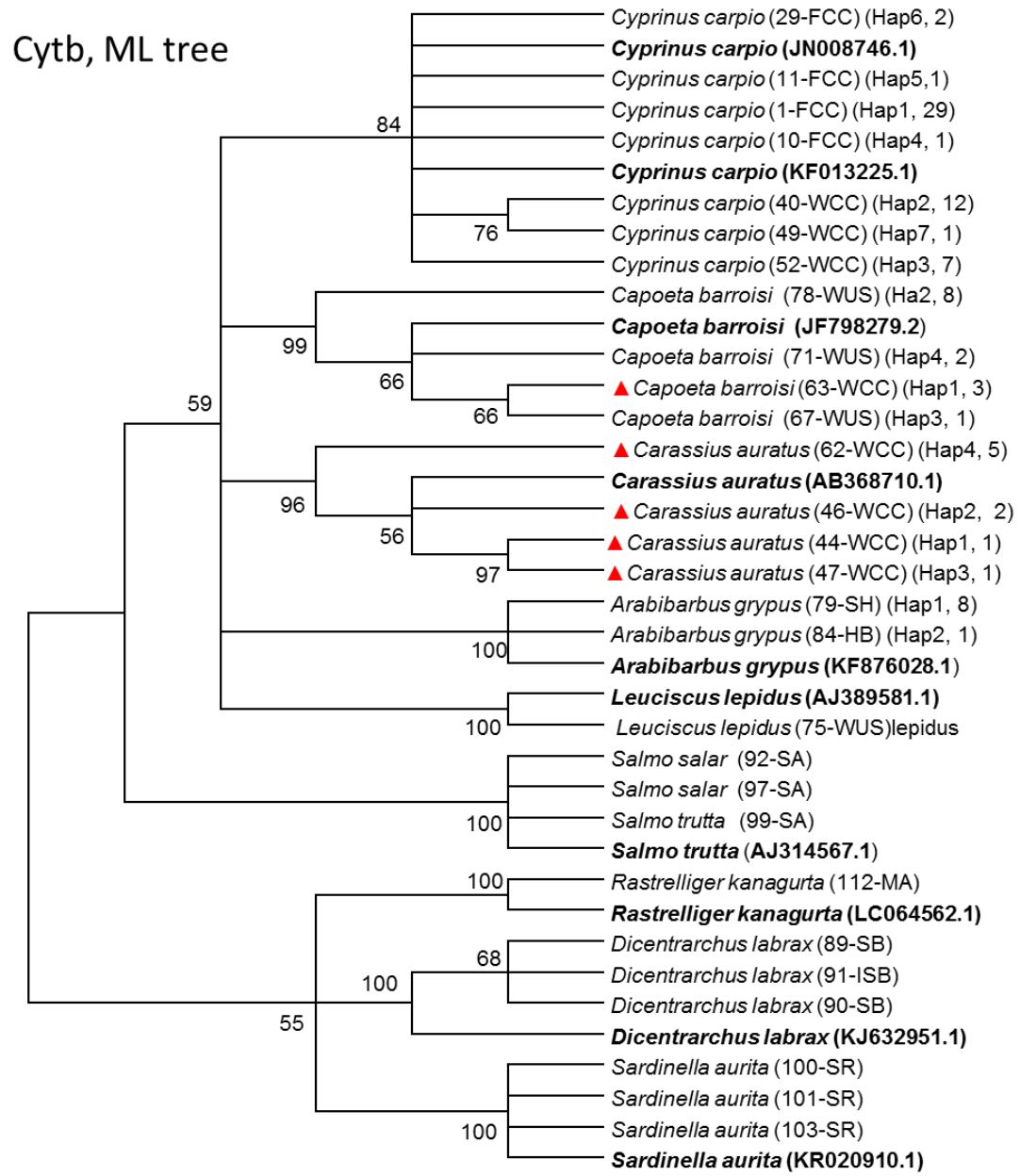
Appendix 4.2: Fish displayed in retail at (a) street markets, (b) fishmongers, and (c) supermarkets in KRI, where labelling can be seen in

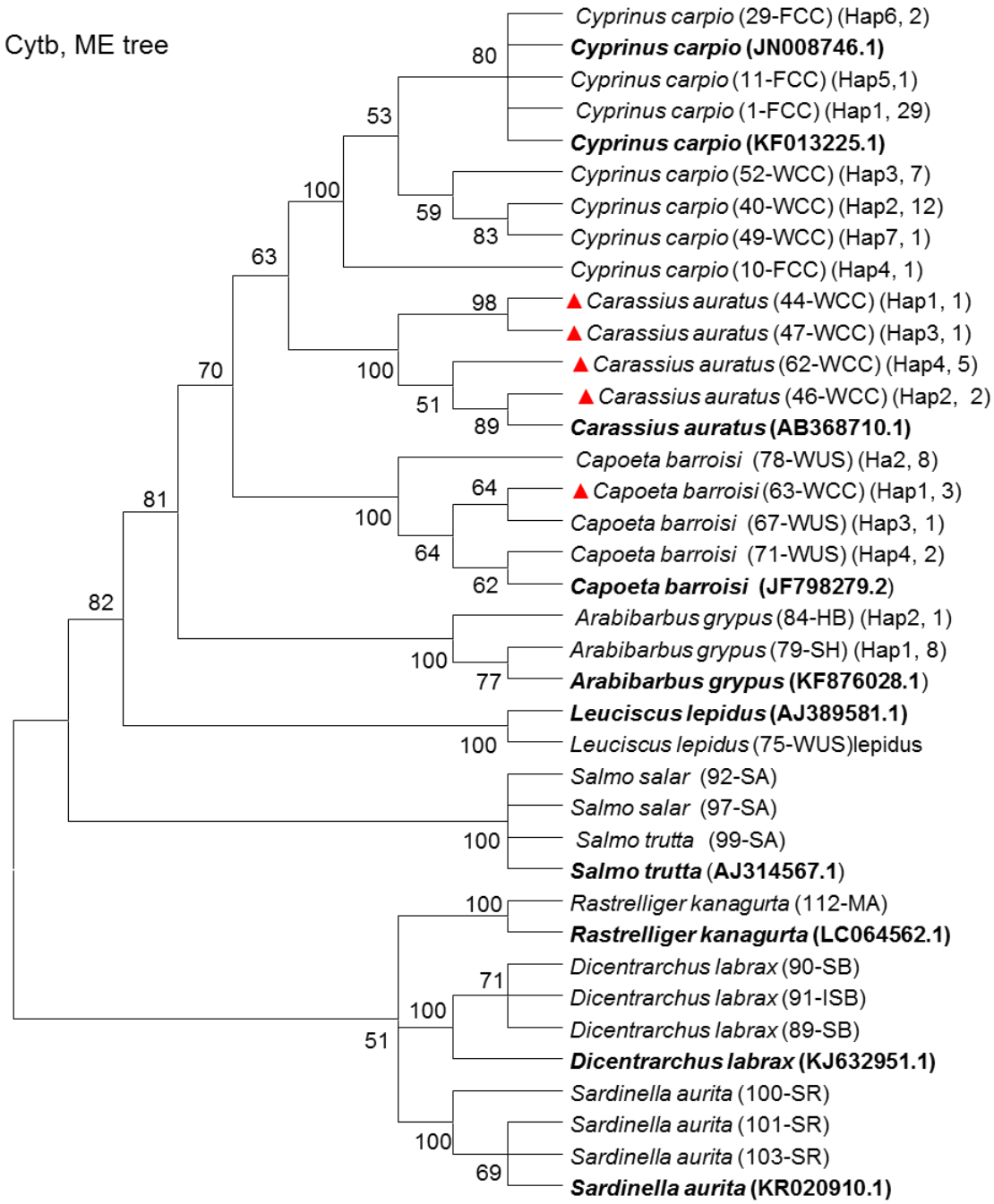
a. Street markets

b. Fishmongers

c. Supermarkets

Appendix 4.3: Different types of trees showing the phylogenetic associations between tested samples. Same conditions in Figure 4.4 and 4.5 were used to construct the trees except for ML tree, the Kimura 2-parameters model was used instead of p-distance. Scale bar used was 0.02. The scale bar corresponds to the number of base substitutions or residue per site




Cytb, ME tree
Cyprinus carpio (29-FCC) (Hap6, 2)
Cyprinus carpio (JN008746.1)
Cyprinus carpio (11-FCC) (Hap5,1)
Cyprinus carpio (1-FCC) (Hap1, 29)
Cyprinus carpio (KF013225.1)
Cyprinus carpio (52-WCC) (Hap3, 7)
Cyprinus carpio (40-WCC) (Hap2, 12)
Cyprinus carpio (49-WCC) (Hap7, 1)
Cyprinus carpio (10-FCC) (Hap4, 1)
Carassius auratus (44-WCC) (Hap1, 1)
Carassius auratus (47-WCC) (Hap3, 1)
Carassius auratus (62-WCC) (Hap4, 5)
Carassius auratus (46-WCC) (Hap2, 2)
Carassius auratus (AB368710.1)
Capoeta barroisi (78-WUS) (Ha2, 8)
Capoeta barroisi (63-WCC) (Hap1, 3)
Capoeta barroisi (67-WUS) (Hap3, 1)
Capoeta barroisi (71-WUS) (Hap4, 2)
Capoeta barroisi (JF798279.2)
Arabibarbus grypus (84-HB) (Hap2, 1)
Arabibarbus grypus (79-SH) (Hap1, 8)
Arabibarbus grypus (KF876028.1)
Leuciscus lepidus (AJ389581.1)
Leuciscus lepidus (75-WUS)lepidus
Salmo salar (92-SA)
Salmo salar (97-SA)
Salmo trutta (99-SA)
Salmo trutta (AJ314567.1)
Rastrelliger kanagurta (112-MA)
Rastrelliger kanagurta (LC064562.1)
Dicentrarchus labrax (90-SB)
Dicentrarchus labrax (91-ISB)
Dicentrarchus labrax (89-SB)
Dicentrarchus labrax (KJ632951.1)
Sardinella aurita (100-SR)
Sardinella aurita (101-SR)
Sardinella aurita (103-SR)
Sardinella aurita (KR020910.1)
80
53
100
59
83
63
98
70
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62
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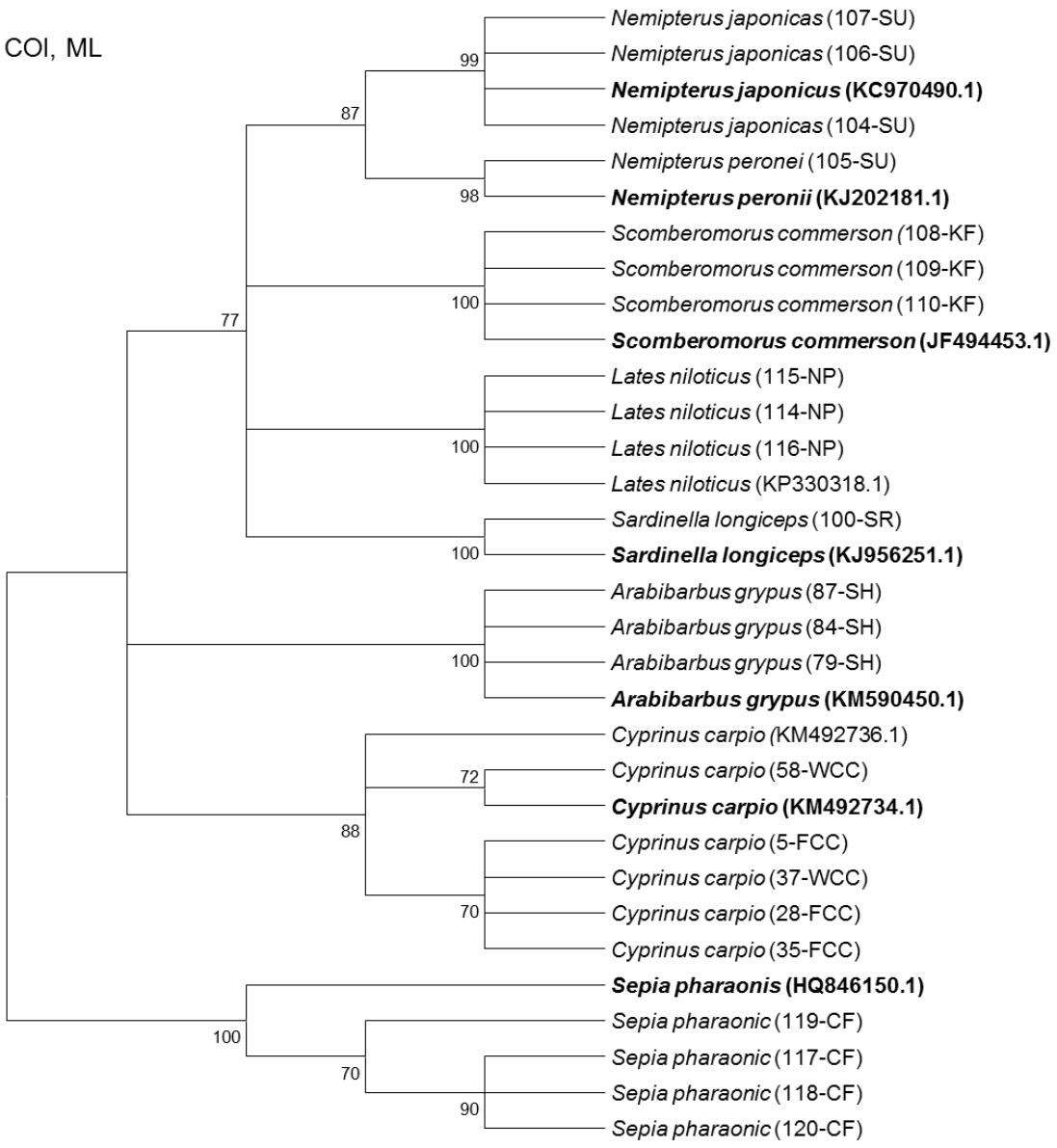
100
77
100
100
100
71
100
51
100
69


COI, ML
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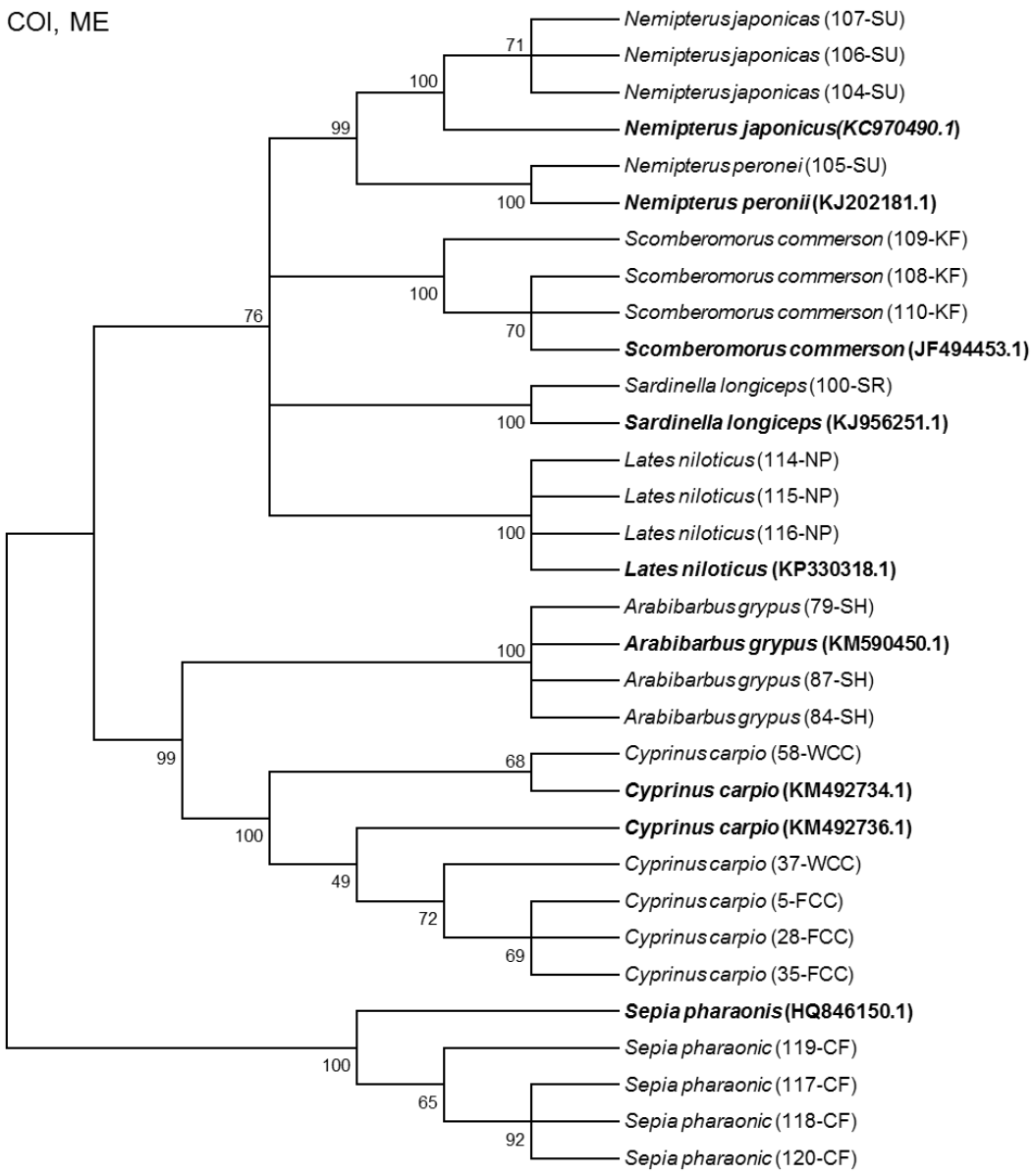
Nemipterus japonicas (106-SU)
Nemipterus japonicus (KC970490.1)
Nemipterus japonicas (104-SU)
Nemipterus peronei (105-SU)
Nemipterus peronii (KJ202181.1)
Scomberomorus commerson (108-KF)
Scomberomorus commerson (109-KF)
Scomberomorus commerson (110-KF)
Scomberomorus commerson (JF494453.1)
Lates niloticus (115-NP)
Lates niloticus (114-NP)
Lates niloticus (116-NP)
Lates niloticus (KP330318.1)
Sardinella longiceps (100-SR)
Sardinella longiceps (KJ956251.1)
Arabibarbus grypus (87-SH)
Arabibarbus grypus (84-SH)
Arabibarbus grypus (79-SH)
Arabibarbus grypus (KM590450.1)
Cyprinus carpio (KM492736.1)
Cyprinus carpio (58-WCC)
Cyprinus carpio (KM492734.1)
Cyprinus carpio (5-FCC)
Cyprinus carpio (37-WCC)
Cyprinus carpio (28-FCC)
Cyprinus carpio (35-FCC)
Sepia pharaonis (HQ846150.1)
Sepia pharaonic (119-CF)
Sepia pharaonic (117-CF)
Sepia pharaonic (118-CF)
Sepia pharaonic (120-CF)
99
87
98
100
77
100
100
100
72
88
70
100
70
90


COI, ME
Nemipterus japonicas (107-SU)
Nemipterus japonicas (106-SU)
Nemipterus japonicas (104-SU)
Nemipterus japonicus(KC970490.1)
Nemipterus peronei (105-SU)
Nemipterus peronii (KJ202181.1)
Scomberomorus commerson (109-KF)
Scomberomorus commerson (108-KF)
Scomberomorus commerson (110-KF)
Scomberomorus commerson (JF494453.1)
Sardinella longiceps (100-SR)
Sardinella longiceps (KJ956251.1)
Lates niloticus (114-NP)
Lates niloticus (115-NP)
Lates niloticus (116-NP)
Lates niloticus (KP330318.1)
Arabibarbus grypus (79-SH)
Arabibarbus grypus (KM590450.1)
Arabibarbus grypus (87-SH)
Arabibarbus grypus (84-SH)
Cyprinus carpio (58-WCC)
Cyprinus carpio (KM492734.1)
Cyprinus carpio (KM492736.1)
Cyprinus carpio (37-WCC)
Cyprinus carpio (5-FCC)
Cyprinus carpio (28-FCC)
Cyprinus carpio (35-FCC)
Sepia pharaonis (HQ846150.1)
Sepia pharaonic (119-CF)
Sepia pharaonic (117-CF)
Sepia pharaonic (118-CF)
Sepia pharaonic (120-CF)
71
100
99
100
100
76
70
100
100
99
68
100
49
72
69
100
65
92

Appendix 4.4: Pairwise P- distances tables for Cytb sequences and COI sequence

Table 4.4A Pairwise P- distances for Cytb sequences. One sample per haplotype was included. All gaps and missing data were eliminated

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1 *Cyprinus carpio* (1-FCC) (Hap1, 29)		0.01	0.00	0.01	0.00	0.00	0.02	0.01	0.01	0.00	0.00	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
2 *Cyprinus carpio* (10-FCC) (Hap4, 1)	0.01		0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
3 *Cyprinus carpio* (11-FCC) (Hap5, 1)	0.00	0.01		0.01	0.00	0.00	0.02	0.01	0.01	0.00	0.00	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
4 *Cyprinus carpio* (16-FCC) (Hap6, 1)	0.02	0.03	0.02		0.01	0.01	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
5 *Cyprinus carpio* (29-FCC) (Hap7, 1)	0.00	0.01	0.00	0.02		0.00	0.02	0.01	0.01	0.00	0.00	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
6 *Cyprinus carpio* (40-WCC) (Hap2, 12)	0.01	0.02	0.01	0.02	0.01		0.01	0.01	0.01	0.00	0.00	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
7 Δ *Carassius auratus* (44-WCC) (Hap1, 1)	0.09	0.10	0.09	0.11	0.09	0.09		0.01	0.00	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
8 Δ *Carassius auratus* (46-WCC) (Hap2, 2)	0.08	0.09	0.08	0.10	0.08	0.08	0.02		0.01	0.01	0.01	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
9 Δ *Carassius auratus* (47-WCC) (Hap3, 1)	0.09	0.09	0.09	0.10	0.09	0.09	0.01	0.01		0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
10 *Cyprinus carpio* (49-WCC) (Hap8, 1)	0.01	0.02	0.01	0.02	0.01	0.00	0.10	0.08	0.09		0.00	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
11 *Cyprinus carpio* (52-WCC) (Hap3, 7)	0.01	0.02	0.01	0.02	0.01	0.01	0.09	0.08	0.09	0.01		0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
12 Δ *Carassius auratus* (62-WCC) (Hap4, 5)	0.08	0.09	0.08	0.10	0.08	0.08	0.02	0.01	0.02	0.08	0.08		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
13 Δ *Capoeta barroisi* (63-WCC) (Hap1, 3)	0.12	0.14	0.12	0.14	0.12	0.12	0.14	0.13	0.13	0.13	0.12	0.13		0.01	0.00	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
14 *Capoeta barroisi* (67-WUS) (Hap3, 1)	0.12	0.13	0.12	0.13	0.12	0.11	0.14	0.13	0.13	0.12	0.12	0.13	0.01		0.00	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
15 *Capoeta barroisi* (71-WUS) (Hap4, 2)	0.11	0.13	0.11	0.13	0.11	0.11	0.13	0.12	0.12	0.12	0.11	0.12	0.01	0.01		0.02	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
16 *Leuciscus lepidus* (75-WUS)	0.16	0.15	0.16	0.17	0.16	0.17	0.16	0.14	0.15	0.17	0.16	0.14	0.21	0.21	0.20		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
17 *Capoeta barroisi* (78-WUS) (Hap2, 8)	0.11	0.12	0.11	0.12	0.11	0.11	0.13	0.12	0.12	0.11	0.11	0.13	0.01	0.01	0.00	0.20		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
18 *Arabibarbus grypus* (79-SH) (Hap1, 8)	0.11	0.12	0.11	0.12	0.11	0.12	0.15	0.13	0.14	0.12	0.12	0.13	0.15	0.15	0.14	0.17	0.14		0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
19 *Arabibarbus grypus* (84-SH) (Hap2, 1)	0.12	0.13	0.12	0.13	0.12	0.12	0.15	0.13	0.14	0.12	0.12	0.13	0.16	0.15	0.15	0.17	0.14	0.00		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
20 *Dicentrarchus labrax* (88-ISB)	0.24	0.25	0.24	0.25	0.24	0.24	0.23	0.22	0.23	0.25	0.25	0.22	0.24	0.23	0.23	0.25	0.23	0.25	0.25		0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
21 *Dicentrarchus labrax* (89-SB)	0.23	0.23	0.23	0.24	0.23	0.23	0.22	0.20	0.21	0.23	0.23	0.21	0.23	0.22	0.22	0.24	0.22	0.24	0.24	0.02		0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
22 *Dicentrarchus labrax* (90-SB)	0.23	0.23	0.23	0.24	0.23	0.23	0.22	0.20	0.21	0.23	0.23	0.21	0.23	0.22	0.22	0.24	0.22	0.24	0.24	0.02	0.00		0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
23 *Dicentrarchus labrax* (91-SB)	0.23	0.23	0.23	0.24	0.23	0.23	0.22	0.20	0.21	0.23	0.23	0.21	0.23	0.22	0.22	0.24	0.22	0.24	0.24	0.02	0.00	0.00		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
24 *Salmo salar* (92-SA) (Hap1, 6)	0.19	0.19	0.19	0.20	0.19	0.20	0.21	0.19	0.20	0.20	0.20	0.20	0.21	0.21	0.20	0.20	0.20	0.18	0.18	0.23	0.23	0.23	0.23		0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02
25 *Salmo salar* (97-SA) (Hap2, 1)	0.19	0.19	0.19	0.20	0.19	0.20	0.21	0.19	0.20	0.20	0.20	0.20	0.21	0.21	0.20	0.20	0.20	0.18	0.18	0.23	0.23	0.23	0.23	0.00		0.00	0.02	0.02	0.02	0.02	0.02	0.02
26 *Salmo salar* (99-SA) (Hap3, 1)	0.19	0.19	0.19	0.20	0.19	0.20	0.21	0.19	0.20	0.20	0.20	0.20	0.21	0.21	0.20	0.20	0.20	0.18	0.18	0.23	0.23	0.23	0.23	0.00	0.00		0.02	0.02	0.02	0.02	0.02	0.02
27 *Sardinella lemuru* (100-SR)	0.21	0.22	0.21	0.22	0.21	0.21	0.22	0.21	0.22	0.21	0.21	0.21	0.21	0.21	0.21	0.24	0.20	0.22	0.22	0.25	0.23	0.23	0.23	0.24	0.24	0.24		0.00	0.00	0.02	0.02	0.02
28 *Sardinella aurita* (101-SR)	0.21	0.22	0.21	0.23	0.21	0.21	0.23	0.21	0.22	0.22	0.21	0.21	0.21	0.22	0.21	0.23	0.21	0.22	0.23	0.24	0.23	0.23	0.23	0.24	0.24	0.24	0.00		0.00	0.02	0.02	0.02
29 *Sardinella lemuru* (103-SR)	0.21	0.22	0.21	0.23	0.21	0.21	0.23	0.21	0.22	0.22	0.21	0.21	0.21	0.22	0.21	0.23	0.21	0.22	0.23	0.24	0.23	0.23	0.23	0.24	0.24	0.24	0.00	0.00		0.02	0.02	0.02
30 *Rastrelliger kanagurta* (111-MA)	0.21	0.21	0.21	0.22	0.21	0.21	0.25	0.23	0.24	0.21	0.21	0.23	0.23	0.23	0.23	0.24	0.22	0.23	0.23	0.27	0.25	0.25	0.25	0.23	0.23	0.23	0.24	0.23	0.23		0.00	0.01
31 *Rastrelliger kanagurta* (112-MA)	0.21	0.21	0.21	0.22	0.21	0.21	0.25	0.23	0.24	0.21	0.21	0.23	0.23	0.23	0.23	0.24	0.22	0.23	0.23	0.27	0.25	0.25	0.25	0.23	0.23	0.23	0.24	0.23	0.23	0.00		0.01
32 *Rastrelliger kanagurta* (113-MA)	0.17	0.17	0.17	0.19	0.17	0.17	0.22	0.20	0.22	0.18	0.17	0.20	0.21	0.22	0.21	0.23	0.21	0.21	0.21	0.27	0.27	0.27	0.27	0.24	0.24	0.24	0.24	0.25	0.25	0.07	0.07	

Table 4.4B. Pairwise P- distances for COI sequences. One sample per haplotype was included. All gaps and missing data were eliminated

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1	*Cyprinus carpio* (58-WCC)		0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02
2	*Arabibarbus grypus* (84-SH)	0.13		0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.01	0.01	0.01	0.01	0.02	0.00	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02
3	*Arabibarbus grypus* (87-SH)	0.13	0.00		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.01	0.01	0.01	0.01	0.02	0.00	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02
4	*Nemipterus japonicus* (104-SI)	0.23	0.22	0.22		0.02	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.02	0.02	0.02
5	*Nemipterus peronei* (105-SI)	0.22	0.22	0.22	0.15		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02
6	*Nemipterus japonicus* (107-SI)	0.23	0.22	0.22	0.00	0.15		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.02	0.02	0.02
7	*Scomberomorus commerson* (108-KF)	0.20	0.21	0.21	0.19	0.19	0.19		0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02
8	*Scomberomorus commerson* (109-KF)	0.21	0.21	0.21	0.19	0.19	0.19	0.00		0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02
9	*Scomberomorus commerson* (110-KF)	0.20	0.21	0.21	0.19	0.19	0.19	0.00	0.00		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02
10	*Lates niloticus* (114-NP)	0.20	0.22	0.22	0.20	0.21	0.20	0.19	0.19	0.19		0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.02	0.02	0.02	0.02
11	*Lates niloticus* (115-NP)	0.20	0.22	0.22	0.20	0.21	0.20	0.19	0.19	0.19	0.00		0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.02	0.02	0.02	0.02
12	*Lates niloticus* (116-NP)	0.20	0.22	0.22	0.20	0.21	0.20	0.19	0.19	0.19	0.00	0.00		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.02	0.02	0.02	0.02
13	*Sepia pharaonic* (117-CF)	0.31	0.30	0.30	0.30	0.32	0.30	0.33	0.33	0.33	0.33	0.33	0.33		0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00
14	*Sepia pharaonic* (118-CF)	0.31	0.30	0.30	0.30	0.32	0.30	0.33	0.33	0.33	0.33	0.33	0.33	0.00		0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00
15	*Sepia pharaonic* (119-CF)	0.30	0.30	0.30	0.31	0.32	0.31	0.32	0.32	0.32	0.33	0.33	0.33	0.01	0.01		0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00
16	*Sepia pharaonic* (120-CF)	0.31	0.30	0.30	0.30	0.32	0.30	0.33	0.33	0.33	0.33	0.33	0.33	0.00	0.00	0.01		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00
17	*Arabibarbus grypus* (79-SH)	0.13	0.00	0.00	0.22	0.22	0.22	0.21	0.21	0.21	0.22	0.22	0.22	0.30	0.30	0.30	0.30		0.02	0.01	0.01	0.01	0.01	0.02	0.00	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02
18	*Nemipterus japonicus* (106-SI)	0.23	0.22	0.22	0.00	0.15	0.00	0.19	0.19	0.19	0.20	0.20	0.20	0.30	0.30	0.31	0.30	0.22		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.02	0.02	0.02
19	*Cyprinus carpio* (5-FCC)	0.02	0.11	0.11	0.22	0.22	0.22	0.19	0.19	0.19	0.19	0.19	0.19	0.30	0.30	0.29	0.30	0.11	0.22		0.00	0.00	0.00	0.02	0.01	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02
20	*Cyprinus carpio* (28-FCC)	0.02	0.11	0.11	0.22	0.22	0.22	0.19	0.19	0.19	0.19	0.19	0.19	0.30	0.30	0.29	0.30	0.11	0.22	0.00		0.00	0.00	0.02	0.01	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02
21	*Cyprinus carpio* (35-FCC)	0.02	0.11	0.11	0.22	0.22	0.22	0.19	0.19	0.19	0.19	0.19	0.19	0.30	0.30	0.29	0.30	0.11	0.22	0.00	0.00		0.00	0.02	0.01	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02
22	*Cyprinus carpio* (37-WCC)	0.03	0.11	0.11	0.22	0.22	0.22	0.19	0.19	0.19	0.19	0.19	0.19	0.30	0.30	0.29	0.30	0.11	0.22	0.00	0.00	0.00		0.02	0.01	0.00	0.00	0.02	0.02	0.02	0.02	0.02	0.02
23	*Sardinella longiceps* (102-SR)	0.21	0.21	0.21	0.22	0.21	0.22	0.18	0.18	0.18	0.20	0.20	0.20	0.35	0.35	0.35	0.35	0.21	0.22	0.20	0.20	0.20	0.20		0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.02	0.02
24	***Arabibarbus grypus* (KM590450.1)**	0.13	0.00	0.00	0.22	0.22	0.22	0.21	0.21	0.21	0.22	0.22	0.22	0.30	0.30	0.30	0.30	0.00	0.22	0.11	0.11	0.11	0.11	0.21		0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02
25	***Cyprinus carpio* (KM492736.1)**	0.03	0.11	0.11	0.22	0.22	0.22	0.19	0.19	0.19	0.19	0.19	0.19	0.30	0.30	0.29	0.30	0.11	0.22	0.01	0.01	0.01	0.01	0.20	0.11		0.00	0.02	0.02	0.02	0.02	0.02	0.02
26	***Cyprinus carpio* (KM492734.1)**	0.02	0.11	0.11	0.22	0.22	0.22	0.19	0.19	0.19	0.19	0.19	0.19	0.29	0.29	0.29	0.29	0.11	0.22	0.01	0.01	0.01	0.01	0.19	0.11	0.01		0.02	0.02	0.02	0.02	0.02	0.02
27	***Lates niloticus* (KP330318.1)**	0.20	0.22	0.22	0.20	0.21	0.20	0.19	0.19	0.19	0.00	0.00	0.00	0.33	0.33	0.33	0.33	0.22	0.20	0.19	0.19	0.19	0.19	0.20	0.22	0.19	0.19		0.02	0.02	0.02	0.02	0.02
28	***Nemipterus japonicus* (KC970490.1)**	0.23	0.22	0.22	0.00	0.15	0.00	0.19	0.19	0.19	0.21	0.21	0.21	0.30	0.30	0.30	0.30	0.22	0.00	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.21		0.02	0.02	0.02	0.02
29	***Nemipterus peronii* (KJ202181.1)**	0.22	0.21	0.21	0.15	0.03	0.15	0.18	0.18	0.18	0.20	0.20	0.20	0.32	0.32	0.32	0.32	0.21	0.15	0.22	0.22	0.22	0.22	0.20	0.21	0.22	0.22	0.20	0.15		0.02	0.02	0.02
30	***Sardinella longiceps* (KJ956251.1)**	0.21	0.21	0.21	0.22	0.21	0.22	0.18	0.18	0.18	0.20	0.20	0.20	0.35	0.35	0.35	0.35	0.21	0.22	0.20	0.20	0.20	0.20	0.00	0.21	0.20	0.19	0.20	0.22	0.20		0.02	0.02
31	***Scomberomorus commerson* (JF494453.1)**	0.20	0.21	0.21	0.19	0.19	0.19	0.00	0.00	0.00	0.19	0.19	0.19	0.33	0.33	0.32	0.33	0.21	0.19	0.19	0.19	0.19	0.19	0.18	0.21	0.19	0.19	0.19	0.19	0.18	0.18		0.02
32	***Sepia pharaonis* (HQ846150.1)**	0.30	0.30	0.30	0.30	0.32	0.30	0.32	0.32	0.32	0.33	0.33	0.33	0.01	0.01	0.01	0.01	0.30	0.30	0.29	0.29	0.29	0.29	0.34	0.30	0.30	0.29	0.33	0.30	0.32	0.34	0.32	

Appendix 5.1: Guidelines for interpretation of R2 values (Williams, 2001)

R^2	Interpretation
Up to 0.5	Cannot use it for NIRS calibration
0.5- 0.70	Poor correlation. Investigation is necessary
0.71- 0.80	Can be used for rough screening. More than 50% of variance in y (NIR data) accounted for by x (reference data)
0.81- 0.90	Can be used for screening and some proximate calibration
0.91- 0.95	Can be used in most applications but with caution. More research is necessary
0.96- 0.98	Can be used in most applications, including quality assurance
0.99+	Can be used in any application

Appendix 5.2: Guidelines for interpretation of RPD value (Williams, 2001)

RPD value	Classification	Application
0.0- 2.3	Very poor	Not recommended
2.4- 3.0	Poor	Very rough screening
3.1- 4.9	Fair	Screening
5.0- 6.4	Good	Quality control
6.5- 6.8	Very good	Process control
8.1+	Excellent	Any application

Appendix A: **Research training and development**

1. Modules training and development

1.1 BIO 5124 (Postgraduate Research Skills and Methods) 3rd October to 10th December 2013

No	Date	Training skills	Facilitator
1	03/10/2013	Field safety and risk assessment	John Eddison
2	18/10/2013	Research ethics and methodology in science	Andy Foey
3	21/10/2013	Research ethics and methodology in science	John Eddison
4	22/10/2013	Laboratory safety and risk assessment	Andy Foey
5	23/10/2013	Good laboratory practice	John Eddison
6	25/10/2013	Written communication	Paul Ramsay
7	05/11/2013	Writing scientific papers	Paul Ramsay
8	06/11/2013	Publishing papers	Paul Ramsay
9	07/01/2014	Biostatistics I	Miguel Franco
10	12/11/2013	Oral presentations	Piero Calosi
11	13/11/2013	Biostatistics Practical	Miguel Franco
12	14/11/2013	Project management and Funding	Tom Hutchinson
13	29/11/2013	Biostatistics II	Miguel Franco
14	05/12/2013	Poster presentations	Rod Blackshaw
15	10/12/2013	Public communication of science	Maria Donkin

1.2 Bio 5102 (Principles and Applications of Electron Microscopy) 25th September to 14th December 2013. Dr Roy Moate

1.3 DIET 107 (Food safety and quality), 1 practical session and 6 lectures, November- December 2012, Dr Victor Kuri and Dr Jane Beal.

1.4 BIOL203/8 (Molecular ecology), 3 practical sessions on fish DNA extraction and identification, November- December 2012 / Dr Mairi Knight.

2. Postgraduate Research Skills & Training Sessions/ Plymouth University

No	Date	Training skills	Facilitator
1	27/11/2013	Introduction to EndNote	Subject Librarians
2	09/01/2013	Introduction to R	Julian Stander
3	20/02/2013	Research Methodology	Martin Coath
4	06/02/2013	SPSS	Luciana Dalla Valle
5	15/03/2013	The Transfer Process	Mick Fuller
6	19/04/2013	Writing for research publication	Chris Wood
7	16/04/2013	Developing professional writing skills for the PhD	Joe Allison
8	15/11/2012	NVIVO	Kevin Meethan
9	29/01/2014	Presenting at conference	Rich Boden
10	26/02/2014	Presenting with confidence	Rich Boden
11	20/05/2014	Meet the editors	Richard Handy
12	22/04/2015	Animal welfare	Stephen Wotten
13	08 /03/2016	Surviving the viva	Heather Skirton
14	10/ 03/2016	Preparing to submit on pearl including copyright and open access	Kate Russel
15	29/03/2016	Transferring your skills into an offer for service	David Karlin
16	01/02/2017	Public health nutrition seminar	Food research collaboration
17	22/03/2017	Postgraduate Networking Event	Sarah Kearns

Appendix A: Membership of Scientific Societies:

- Institute of Food Science and Technology (IFST)
- The Society of Food Hygienic and Technology (SOFHT)
- FRC (Food Research Collaboration)

Appendix B: Conferences, workshops, and courses attended

No	Date	Event	Venue
1	11/ 03/2013	The Postgraduate Society Conference Series	Plymouth University
2	18 /06/2013	The Postgraduate Society Conference Series	Plymouth University
3	19/06/2013	CARS Postgraduate Symposium	Duchy College, United Kingdom
4	06/06/2014	CARS Postgraduate Symposium	Rothamsted Research, Oakhampton, UK
5	26-29/06 /2013	Hands on Molecular Biology workshop	Plymouth University
6	01-03/07 /2013	Hands on Proteomics Workshop	Plymouth University,
7	19/03/2014	The Postgraduate Society Conference Series	Plymouth University
8	14-15/05 /2014	Institute of Food Science and Technology/Jubilee Conference,	London, United Kingdom
9	09/07/2014	Electron Microscopy Event	Plymouth University
10	25-28/11 /2014	28th EFFoST International Conference/ 7th International Food Factory for the Future Conference	Uppsala, Sweden
11	24/03/2015	Postgraduate Society Conference	Plymouth University
12	18/06/2015	Food fermentation workshop/ (Victor Kuri and Colin Trier)	Plymouth University
13	23/06/ 2015	The Postgraduate Society Conference Series	Plymouth University
14	02/12/2015	The Postgraduate Society Conference Series	Plymouth University
15	20/01/2016	Joint Research Group Conference, School of Biological Sciences	Plymouth University
16	29/04/2016	Transferring your skills into an offer for services	Plymouth University
17	6-8/07/2016	ISEKI 2016 Food conference	Vienna, Austria
18	15/03/2017	Postgraduate Society Research Showcase	Plymouth University
19	09/06/2017	School of Biological and Marine Sciences Poster, Presentation and Networking Event	Plymouth University

Appendix C: Award

BIOLOGICAL SCIENCES WITH PLYMOUTH UNIVERSITY

JOINT RESEARCH GROUPS CONFERENCE PRIZE 2016

This certificate is awarded to

SALIH SALIH

for the best Post-graduate research poster

SCHOOL OF BIOLOGICAL SCIENCES, PLYMOUTH UNIVERSITY

Signature

20th Jan 2016

Date

Appendix D: Food Safety Certificate

Chartered Institute of Environmental Health

Level 2 Award in Food Safety in Catering

1 credit

Salih Mustafa Salih

has successfully completed a programme of training and an assessment which concluded the course

Course Director

Chief Executive
Chartered Institute of Environmental Health

Graham Jukes

Examination Date: *09 November 2012*

CIEH recommends you refresh your training by *09 November 2015*

Centre number: *23168*

Certificate number: 8872503

Original Issue Date: *22 November 2012*

Issue number: 1

PASS

Qualification accreditation number – 500/5476/4
Accredited only for England, Wales and Northern Ireland

Ofqual

Llywodraeth Cynulliad Cymru
Wales Assembly Government

cea
Rewarding Learning

Appendix E: Posters presented

EFFoST 28th EFFoST Conference 25-28 November 2014 Uppsala Konsert and Kongress, Uppsala, Sweden

Effect of inulin inclusion on the physiochemical, eating quality, and cooking properties of doner kebab

Salih M. Salih, Calvin Hopkins and Victor Kuri
School of Biological Sciences, Plymouth University, PL4 8AA, UK
Salih.salih@plymouth.ac.uk

RESEARCH WITH PLYMOUTH UNIVERSITY

Introduction

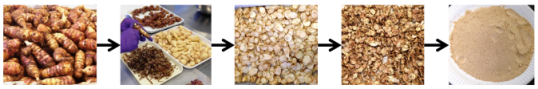
Doner kebabs are a fast food expanding in popularity and availability, but they are commonly high in fat, and deficient in fibre content. Because, conscious consumers are increasingly aware of the links between excessive consumption of processed meats and poor health, a need to modify the formulations was perceived. Addition of fibre (inulin) could be used as fat replacement on certain foods, but the acceptability needed to be considered

Objective

AIM: To investigate the effect of addition of inulin as fat replacement on the physicochemical, sensory properties and overall acceptability of doner kebab meat and to assess the effect of chopping degree on the cooking properties and texture

Methodology

Materials: lean lamb meat, fatty lamb meat, water, (extracted oil spices and salt 0.5%), commercial inulin (CI) marketed as TEX from chicory roots and a prepared powder (flour) from tubers of (*Helianthus tuberosus)* Jerusalem artichoke (JA) (figure 1).



Fresh tubers Peeled tubers Sliced tubers Dried slices Powder of JA

Figure 1: The processing steps of making powder from JA

Table 1: Product's formulation

Degree of chopping	Batch	Ingredients proportions (%)					Fat content (%)
		Meat ingredients		Non-meat ingredients			
		Lean meat	Fatty tissue	Water	Inulin powder	Inulin gel	
Fine chopping	CP	34.5	65.5	------	--------	------	40
	7% JA.P	59.75	20.25	13	7	------	20
	7% JA.G	59.75	20.25	0	-------	20	20
	7% CI.P	59.75	20.25	13	7	------	20
	7% CI.G	59.75	20.25	0	--------	20	20
	5% JA.G	59.75	20.25	6	-------	14	20
	5% CI.G	59.75	20.25	6	-------	14	20
Very fine chopping	7 % JA.P	59.75	20.25	13	7	------	20
Coarse chopping	7% JA.P	59.75	20.2	13	7	------	20

Factors studied

- Source of inulin (commercial inulin, and Jerusalem artichoke flour)
- Levels of inulin addition (5% and 7%)
- Incorporation method (powder (P) and gel(G)
- Degree of chopping (coarse, fine, and very fine chopping

Tests performed

- Chemical analysis (moisture, ash, protein and lipid)
- Gross energy, pH, water activity
- Instrumental texture (TPA, shear force) and colour ($L^* a^* b^*$)
- Cooking properties (cooking yield, fat and water retention)
- Sensory evaluation

Results

Table 2: Proximate composition (g/100g) and energy values of cooked doner samples (mean± SD)

Batch	Moisture	Ash	Protein	Fat	CHO's*	Energy (Kcal/10g)
CP×	50.3±1.09^{bc}	2.7±0.60^{a}	27.3±0.81^{a}	19.0±0.72^{d}	0.5	319.36
7% JA.P	52.0±1.7^{abc}	2.4±0.55^{ab}	18.8±0.30^{d}	18.8±0.14^{d}	7.7	302.22
7% JA.G	52.6±1.01^{abc}	2.3±0.63^{ab}	18.9±0.10^{de}	18.9±0.10^{d}	7.1	301.94
7% CI.P	49.9±2.32^{bc}	1.6±0.75^{abc}	21.0±0.816^{b}	21.6±0.20^{b}	5.7	346.21
7% CI.G	48.6±1.48^{c}	0.8±0.47^{c}	19.4±0.62^{bc}	25.3±0.82^{a}	5.6	332.82
5% JA.G	54.3±0.65^{abc}	1.6±0.59^{abc}	19.1±0.66^{d}	20.8±1.14^{bc}	4.1	298.77
5% CI.G	56.2±3.84^{a}	1.1±0.29^{bc}	17.3±0.11^{e}	20.1±0.33^{bcd}	4.9	291.75
7% JA.P	55.5±0.86^{ab}	1.8±0.26^{abc}	20.8±0.09^{bc}	13.0±0.25^{e}	8.7	254.21
7% JA.P	51.6±2.79^{abc}	2.0±0.28^{abc}	18.5±0.50^{de}	19.2±0.11^{bc}	8.5	304.29

$^{a-e}$Mean ± standard deviations (n=3) in the same column with different superscripts are significantly different (P <0.05). * Carbohydrates, ×Control product

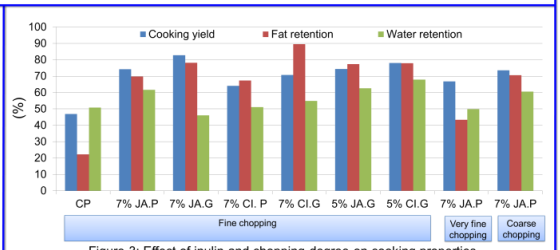


Figure 3: Effect of inulin and chopping degree on cooking properties

Table 3: Effect of inulin on textural parameters and shear force

Batch	Hardness (kg)	Springiness	Cohesiveness	Gumminess (kg)	Chewiness (kg x cm)	Shear force (Kg)
CP	2.18±0.13^{ab}	0.77±0.07^{a}	0.54±0.05^{a}	1.17±0.11^{ab}	0.92±0.16^{ab}	5.27±0.93^{a}
7% JA.P	2.94±0.58^{a}	0.77±0.04^{a}	0.51±0.01^{a}	1.52±0.32^{a}	1.18±0.21^{a}	3.76±0.45^{ab}
7% JA.G	1.79±0.32^{b}	0.81±0.06^{a}	0.51±0.02^{a}	0.92±0.12^{bc}	0.74±0.04^{ab}	4.34±1.16^{abc}
7 % CI.P	1.94±0.52^{ab}	0.80±0.05^{a}	0.51±0.03^{a}	0.99±0.28^{abc}	0.80±0.23^{ab}	2.75±0.58^{bcd}
7% CI.G	2.19±0.53^{ab}	0.74±0.03^{a}	0.49±0.03^{a}	1.09±0.34^{abc}	0.82±0.30^{a}	3.35±0.28^{bcd}
5% JA.G	1.97±0.10^{ab}	0.83±0.05^{a}	0.52±0.02^{a}	1.04±0.07^{abc}	0.87±0.11^{a}	2.16±0.38^{bc}
5% CI.G	1.15±0.28^{b}	0.77±0.01^{a}	0.51±0.02^{a}	0.58±0.12^{c}	0.45±0.08^{b}	1.84±0.61^{cd}
7 % JA.P	2.01±0.24^{ab}	0.83±0.02^{a}	0.53±0.01^{a}	1.07±0.11^{abc}	0.89±0.07^{ab}	2.91±0.55^{cd}
7% JA.P	2.14±0.24^{ab}	0.75±0.007^{a}	0.49±0.02^{a}	1.05±0.06^{abc}	0.80±0.04^{ab}	2.16±0.34^{d}

Values are given as mean ± standard deviations, with different letters $^{a-c}$ within the column are significantly different (P < 0.05).

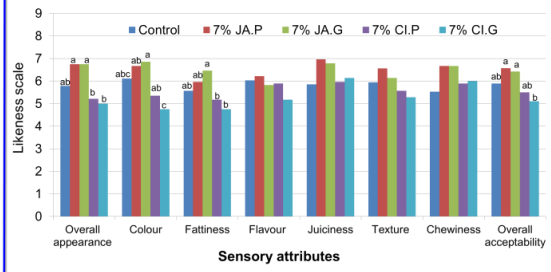


Figure 4: Sensory evaluation of doner kebab based on hedonic 9 points scale
Values (n=28) with different letters $^{a-c}$ within each attributes are significantly different (P < 0.05)

Conclusions

- Dramatic improvement of cooking properties in all batches (Figure 3) by inulin compared to the control
- Generally JA samples had higher cooking yields than CI samples especially at 7% level
- Textural properties were slightly modified
- Softer and less chewy product resulted from JA-gel than from JA-powder or control samples
- Shear force was decreased by inulin, resulting in more tender product
- Better cooking properties were obtained with fine and coarse chopping when compared to very fine chopping
- Chopping degree had no significant effect (p> 0.05) on texture parameters
- pH value increased significantly (p<0.05) in cooked samples with added inulin
- Most treatments were generally acceptable for most sensory attributes, but samples with 7% of JA -gel or powder scored the highest for acceptability.
- To conclude, JA had a few advantages over the CI ingredient

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Fish labelling at commercial markets in Kurdistan Region-Iraq: Application of DNA barcoding for fish authentication

Salih Salih, Carly Benefer, Mairi Knight, and Victor Kuri
School of Biological Sciences, Plymouth University, PL4 8AA, UK
Salih.salih@plymouth.ac.uk

RESEARCH WITH PLYMOUTH UNIVERSITY

Introduction

- Fish authenticity is a growing issue in the global markets
- Large price differences between species and between farmed and wild fish results in profitable mislabelling fraud
- Several studies have revealed deliberate mislabelling, where valuable fish are substituted with those of lower value, in a greater range of species, markets and countries
- Currently, there are about 70 types of fish species available in Iraqi waters.
- Carp, in particular common carp (*Cyprinus carpio),* is one of the most important food sources for human consumption in Kurdistan Region-Iraq (KRI)
- Due to the lack of labelling enforcement in Kurdistan Region, consumers are concerned with the accuracy of fish labelling

Objectives:

- To use DNA barcoding to identify fish bought in Kurdistan markets in order to detect potential mislabelling issues
- To determine whether DNA barcoding can be used to distinguish between wild and farmed common carp

Materials and Methods

- A total of 120 fish samples (33 imported and 87 local carp) were purchased from different commercial markets in Erbil province/ KRI during Dec2014- Jan15.
- Total genomic DNA was isolated from dorsal muscle tissue followed a rapid and inexpensive HotSHOT method (1).
- The Cytochrome b (Cytb) region was amplified in all samples using primer set H15149/L14735 (2), but where no identification could be made at this region, primers FishF1_t1/FishR1_t1 (3) targeting the Cytochrome c oxidase subunit I (COI) gene were used.
- Purified PCR products were sent to Macrogen Inc. for sequencing with the forward and reverse directions.
- For molecular identification the sequences were compared to the Basic local alignment search tool (BLAST) and/ or Barcode of Life Database (BOLD)
- Sequences were aligned using ClustalW, haplotypes were calculated (for Cytb only) using DnaSP v.5 (4) and neighbour joining trees produced in MEGA6 (5) (Figure 1).

Results

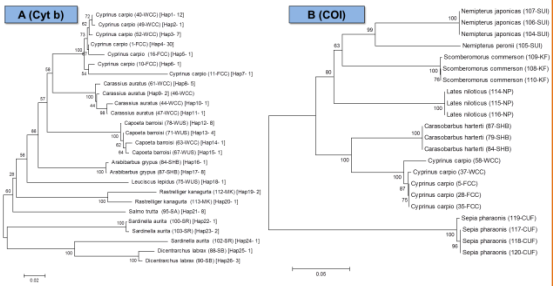

Figure 1. Neighbour joining (NJ) tree constructed in MEGA 6 for (A) Cytb gene sequences (1 sequence from each of 26 haplotypes with 340pb) and (B) COI gene sequences (22 sequences of 559bp) . Hap= Haplotype, CUF= Cuttlefish, KF= kingfish, FCC= Farmed common carp, WCC= Wild common carp, WUS= Wild unknown species, SHB= Shabbout, SA= Salmon, MA= Mackerel, SR= Sardine, SB= Sea bass, SUI= Sultan ibrahim, NP= Nile perch.

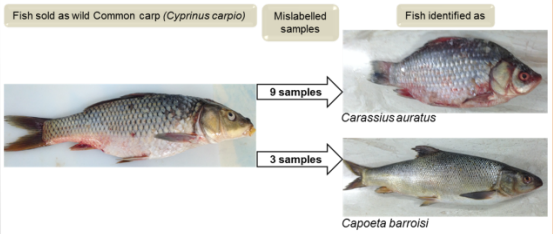

Figure 2. Graphical summary of the mislabelling of whole fresh fish in Kurdistan markets found in this study.

Table 1. List of all collected samples (n=120) with declared names matched to the maximum identity using Cytb and COI genes.

Fish declared as (common name)	No. of samples	Process status	Purchased locations	Price (£/kg)	Maximum identity (%)			Mislabelling
					Cytb	COI		
					BLAST	BLAST	BOLD	
Common carp, wild	31	Fresh, whole	Fishmongers, Street markets	6-8	99-100	99[a]	99-100[a]	Yes, 12 samples (Figure 2)
Common carp, farmed	27	Fresh, whole	Farmers, fishmongers	4-5	99-100	99[a]	100[a]	No
Common carp, farmed	8	cooked	Restaurants	8-10	99-100	---	---	No
Shabbout	9	Fresh, whole	Fishmonger	9-10	99-100	Insufficient match[a,b]	Sequence not available	No
Wild unknown species	12	Fresh, whole	Street markets	5-6	99-100	---	---	N/A
Salmon	4	Filleted	Supermarkets	16-17	99-100	---	---	No
Salmon	4	Frozen	Supermarkets	14-15	99-100	---	---	No
Sea bass	4	Fresh, whole	Supermarkets	11-12	98-99	---	---	No
Sardine	4	Fresh, whole	Supermarkets	10-11	98-100	---	---	No
Mackerel	3	Fresh, whole	Supermarkets	12-14	98-100	---	---	No
Sultan Ibrahim fish	4	Fresh, whole	Supermarkets	11-12	Insufficient match[b]	98-100	99-100	No
Nile Perch	3	Filleted	Supermarkets	14-16	Insufficient match[b]	99-100	100	No
Kingfish	3	Filleted	Supermarkets	16-17	Insufficient match[b]	99-100	100	No
Cuttlefish	4	Cubes	Supermarkets	12-14	Insufficient match[b]	99	98-100	No

[a]For a selection of only three samples, [b]Insufficient match = less than 97%.

Discussion and conclusions

- The COI gene alone was initially trialled across a range of samples, but due to poor PCR amplification and some non-specific bands, Cytb was used as the premier method.
- Only 14 sequences did not match those in public sequence databases with the Cytb gene, possibly due to poor DNA quality and/ or contamination and were successfully sequenced with COI instead. No COI barcode sequence for Shabbout (*Carassius auratus*) was available in BOLD.
- All carp species were successfully sequenced at the Cytb region with fresh whole, cooked and filleted fish, while COI was more reliable with imported species.
- In both of the NJ trees (Figure 1) most of the samples were grouped with other samples of the same species, implying the sequence data and identifications were robust.
- The rate of mislabelling was about 10% in this study and all of these cases were found in wild common carp - wild fish have premium prices over farmed common carp.
- In the survey, 9 goldfish (*Carassius auratus*) and 3 *Capoeta barroisi samples* were sold as wild common carp and detected as mislabelling cases (Figure 2).
- Goldfish is closely related to common carp (6) and have a similar morphological appearance, therefore this mislabelling could be a case of accidental rather than deliberate fraud.
- Mislabelling occurred at street markets and fishmongers, while in supermarkets no mislabelling was detected (these were mostly imported fish).
- Wild and farmed common carp were not discriminated by this method as there was no clear grouping based on wild/ farmed (Figure 1), however this may possibly due to the fact that farmed fish may sold as wild (economic gain), but other genetic or chemical methods e.g. multi-element profile and Near Infrared Spectroscopy (NIR) are applying to discriminate between wild and farmed common carp.
- Generally, a lack of regulation and enforcement is one of the main contributions for seafood mislabelling (7) but here it is likely due to mistaken identification rather than deliberate fraud.

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- To Co-author Carly Benefer for her support on the molecular work
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Applications of multi-element fingerprinting and near-infrared spectroscopy for the authentication origin of wild and farm common carp (*Cyprinus carpio)*

Salih Salih and Victor Kuri
School of Biological Sciences, Plymouth University, PL4 8AA, United Kingdom
salih.salih@plymouth.ac.uk

RESEARCH WITH PLYMOUTH UNIVERSITY

ISEKI_Food 2016

Introduction

- Assessment of fish origin whether wild or farmed is a measure to protect consumers and avoid fraud
- Common carp (*Cyprinus carpio*) as a freshwater fish species, has been one of the most cultivated and popular fish species breed all over the world
- Common carp is the main cultured fish species in Iraq, first introduced to the country in 1950s, and farmed from the 1960s
- Aquaculture in Kurdistan Region of Iraq (KRI) has grown steadily over the past two decades becoming an important source of food
- The majority of wild fish species have premium prices over farmed fish
- A question raised in consumers' minds is whether the nutritional value and safety concerns of farmed fish is equivalent to that of wild fish[1,2]
- Near-infrared (NIR) spectroscopy emerged as rapid, simple, non-destructive technique for quantifying food components and could be used as the basis for authentication of fish origin[3,4]

Aim

To investigate the applications of NIR for the authentication origin of wild and farmed common carp based on composition of muscle fish (minerals and chemical composition)

Materials and Methods

Samples collection and preparation

- Fresh fish samples (n=56) declared as wild (n=29) or farmed (n=27) common carp were purchased from different commercial markets in Erbil province/ KRI during Dec2014- Jan15.
- Following fish skin removal, filleting and, washing, muscle portions (250-300g) were freeze-dried, grinded and homogenized. All measurements were taken in triplicate

Chemical analysis

- The chemical composition (moisture, protein, ash and lipid) of 25 samples were determined using AOAC protocol as reference (measured values)
- The minerals contents [K, P, S, Na, Ca, Mg, Zn, and Fe (mg/g)] of all samples were determined on freeze-dried muscle by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) as reference samples (measured value).

NIR analysis

- All homogenized dried samples (~20g) were scanned in reflectance mode at 2 nm intervals over NIR spectral wavelengths from 2850 to 800 nm using scanning monochromator instrument (Matrix-I, Bruker Optic Gmbh, Germany). A spectrum for each sample represented the average of 64 multiple scans
- Chemometrical software OPUS^{TM} QUANT of Bruker (Version 6) was used for computing the models and for quantitative analysis
- The calibration models were developed from reference samples with their spectra separately for each of the minerals (n=32) and chemical composition (n=25) using partial least squares (PLS) regression method. To optimize calibration accuracy, spectra were subjected to a variety of pre-processing transformations included; vector normalization, multiplicative scatter correction, minimum and maximum normalization. The full cross validation (leave-one-out) was used to evaluate the developed models.
- Same set of samples were used for both calibration and validation. Principal component analysis (PCA) was used for classification of origin and species (Figure 2)

Results

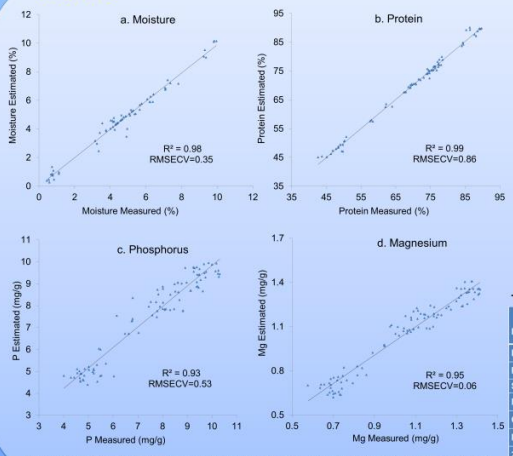

Figure 1. Measured values and estimated value of A (Moisture), B (Protein), C (P), and D (Mg)

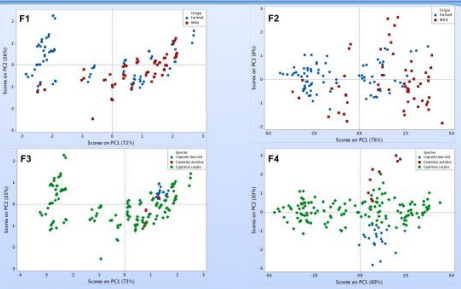

Figure 2. PCA score plot of the estimated NIR data based on minerals and chemical composition: (F1 and F3) chemical composition, (F2 and F4) minerals

Table 1. Estimated data by NIR (E1)minerals' concentration (mg/g) (E2) chemical composition (%) in muscle fish

(E1) Minerals	*Cyprinus carpio* Wild (n=19)	*Cyprinus carpio* Farmed (n=27)	*Capoeta barroisi* (n=7)	*Carassius auratus* (n=3)
K	14.85±3.01	11.26±3.25	14.05±0.57	13.24±1.25
P	8.34±1.61	6.44±1.93	8.14±0.58	7.39±0.73
S	8.13±0.93	6.43±1.35	8.03±0.59	8.27±0.35
Na	2.29±0.68	1.58±0.53	2.95±0.33	1.72±0.50
Ca	2.12±0.82	1.44±0.68	2.24±0.79	1.43±0.30
Mg	1.18±0.24	0.87±0.21	1.14±0.05	1.11±0.05
Zn	0.042±0.014	0.028±0.007	0.026±0.004	0.056±0.008
Fe	0.037±0.01	0.032±0.001	0.028±0.002	0.046±0.013

(E2) Component	*Cyprinus carpio* Wild (n=19)	*Cyprinus carpio* Farmed (n=27)	Capoeta barroisi (n=7)	Carassius auratus (n=3)
Moisture	3.57±0.66	3.26±2.27	1.80±2.16	5.99±0.76
Protein	81.11±11.30	60.72±14.68	79.39±2.97	71.61±2.81
Fat	13.19±11.19	30.89±16.20	11.69±2.11	14.57±0.63
Ash	4.40±1.12	4.27±1.11	5.73±0.23	4.7±0.64

Discussion and conclusions

- Accumulation patterns show a tendency for higher levels of most of the analysed minerals in wild fish. However, there were no statistically significant differences between wild and farmed fish as well as between different species (Table 1. E1)
- Farmed samples tend to have higher lipid and lower protein contents (Table 1. E2)
- Good prediction performance, indicated by coefficients of determination (R^2) and root mean square errors of cross validation (RMSECV) was obtained for [moisture (97.5, 0.35), protein (99.4, 0.86) (Figure 1)], fat (99.5, 0.97) ash (96.0, 0.76), and for some of the minerals: [P (92.5, 0.53), Mg (94.6, 0.06)(Figure 1)], S (94.0, 0.31), K (90.5, 1.06). While for a few minerals, the prediction models were not satisfactorily accurate.
- The results confirmed that NIR spectroscopy is a useful technique for predicting of macro-mineral contents and chemical composition (moisture, protein, lipids and ash)
- Results demonstrate that the multi-element fingerprinting using NIR data may contribute to discriminate sample groups, but not for authentication when used alone
- Although there was no clear indication of the discrimination using PCA between wild and farmed common carp, the majority of wild common carp were in the right side of the plot and a few of wild were misclassified in the left side especially with chemical composition (Figure 2, F1). Therefore, this misclassification of some wild fish could be a cases of mislabelling
- The classification of the three fish species was obvious, where all *carassius auratus* were in the positive side of PC1, and most of the *capoeta barroisi* were in the negative side of PC1. All the *Cyprinus carpio* (wild and farm) were spread over the two components (Figure 2, F3 and F4)

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- To the College of Agriculture- Depts. of Food Technology and Fish Resource and Aquatic Animals at Salahaddin University-Hawler/ Kurdistan Region for providing laboratory facilities to collect and prepare fish samples

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