HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

APPLICATION OF ELECTRONIC TONGUE AND NEAR INFRARED SPECTROSCOPY TO DETECT ADULTERATION OF SOME FOODS WITH HIGH ECONOMIC VALUE

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LIST OF ABBREVIATIONS

1st Derivative	First derivative Savitzky–Golay filter (21 points)
2nd Derivative	Second derivative Savitzky–Golay filter (21 points)
Benchtop Spectrophotometer	MetriNIR (MetriNIR Research, Development and Service Co., Budapest, Hungary)
EC	European Commission
E-tongue	Electronic tongue
EU	European Union
E_varX	Explained variance of X variables
E_varY	Explained variance of Y variables
FDA	Food and drug administration
g	Gram
G.M.C	Grape must concentrate
Handheld Spectrophotometer	NIR-S-G1 (InnoSpectra Co., Hsinchu, Taiwan)
kg	Kilogram
LDA	Linear discriminant analysis
LDPE	Low density polyethylene plastic
LOD	Limit of detection
LOO	Leave-one-out cross-validation
LOQ	Limit of quantification
MSC	Multiplicative scatter correction
NIRS	Near infrared spectroscopy
PCA	Principal component analysis
PC's	Principal components
PLSR	Partial least square regression
RMSEC	Root mean square error of calibration
RMSECV	Root mean square error of cross-validation
RMSEP	Root mean square error of prediction
R^2pred	Determination coefficient of prediction
R^2C	Coefficient of determination
R^2CV	Coefficient of cross-validation
Sgolay	Savitzky–Golay filter (21 points)
SNV	Standard normal variate
w/w	Weight per weight
w/v	Weight per volume

1. INTRODUCTION

Food quality is an important aspect of the food industry that has gained increased attention with the continuous development of living standards and the relative change of dietary structure. There is a rising and persistent increase in demand for nutritious and safe food owing to the recent surge in fraudulent activities in the food industry, especially for foods with high economical and global value such as wine, protein powders and meat. Tokaji wine for instance is a Hungarian delicacy acknowledged for its unique taste, which is believed to be acquired from the noble rotten berries (botrytized berries) used during its production. Exclusion of botrytized berries during Tokaji wine production or manipulating the sugar content of lower grade wines to imitate high grade wines are some of the suspicions fraudulent activities that threaten the hard-earned reputation of Tokaji wines. In the protein powder industry, conventional analytical methods measure total nitrogen, not specifically protein nitrogen. That means certain adulterants with high nitrogen content or non-organic sources can be used to make an apparent increase in the pseudo protein content of protein powders for a competitive edge and financial gain. These new trends of adulteration in most instances, are to maneuver detection when conventional methods are used. In the meat industry, quality assessments are often a rather complex concept, which includes different microbiological, physicochemical and biochemical attributes (Woodvine 2009) which can all be tampered with at any point in the food production chain for financial gains.

The great variability in food processing methods, thus, leads to a high variability in many products on the market and imposes great pressure on the food manufacturing industry to explore new emerging technologies for reliable product monitoring. Quality assurance and quality control are major tasks in the production and processing of foods that are often related to consistency in chemical and sensory attributes (Haddi et al. 2015). In the wine, protein powder and meat industry, many compositional characteristics come into play in implementing quality control. Standard analytical techniques (conventional methods) used for monitoring such parameters include the Kjeldahl method (for crude protein), Soxhlet Extraction Method (for crude fat), pH meter (acidity/alkalinity), colorimeter (for color) (Pugliese and Sirtori 2012). Some of these methods however, are tedious, destructive, time consuming and in some cases expensive and often do not give a complete overview of the food being investigated.

Challenges such as these have led to the exploration of rapid alternatives that are capable of overcoming these setbacks. Major examples of such alternatives are spectroscopy, an interaction between light and an analyte in a defined wavelength range that overlays a compositional overview of the analyte. In the near-infrared (750-2500nm) region, the technique can provide information through vibrational bonding in the form of overtones and combination

bands, which can be interpreted with advanced statistical tools. In spite of the low intensity, the band shape often defines a single compound or group of compounds, making this technique suitable for qualitative and quantitative purposes for food authentication. Near infrared spectroscopy (NIRS) has been widely used in the wine industry for monitoring different food parameters ranging from grape quality to wine characterization (Aleixandre-Tudo et al. 2018; Petrovic, Aleixandre-Tudo, and Buica 2020; Osmond et al. 2010) but with a paucity for tracking wine adulteration particularly, wine adulteration with sweeteners such as grape concentrate. Recent studies show that it has also, been used to monitor protein powder quality in diverse forms (Osborne 2000; DeVries et al. 2017; Lukacs et al. 2018) but complex forms of adulteration with innovative nitrogenous based substances at low concentration have not been reported. In addition, the emergence of handheld devices capable of remote analysis could also be explored for practical quality control purposes.

Another trend in the food industry for quality control is the adoption of sensory evaluations. Trained panels are often utilized in quality control cases or in product development projects in the initial phase (J. Yang and Lee 2019). Sensory evaluation is centered on the proper functioning of the five human senses (vision, sound, touch, smell and taste). Although screening is often performed to improve sensory results, the human senses are often prone to fatigue during long periods of sensory evaluation. In addition, the process is subjective, can be time consuming and could pose some significant risk to the assessor depending on the food content and the human senses that are being used (Maria Sirangelo 2019). Challenges such as these propelled interest in electronically engineered alternatives for food monitoring. A common example is the electronic tongue (e-tongue). The International Union of Pure and Applied Chemistry (IUPAC) defines the electronic tongue as “a multisensory system consisting of a number of selective sensors and uses advanced mathematical procedures for signal processing based on pattern recognition and/or multivariate data analysis” (Vlasov et al., 2005). In comparison with the human tongue, e-tongue has improvements in the sensitivity, selectivity, and multiplexing capacity of modern biosensors (Perumal and Hashim 2014). It is capable of providing rapid, real-time, accurate and reliable data about various samples understudy and has gained fame in the pharmaceutical, cosmetics, environmental control, engineering (petroleum), agriculture, food beverage industries.

For quality control purposes, e-tongue has been applied for the geographical authentication of monofloral honey (Wei and Wang 2011a), Moroccan honey (El et al. 2017), Hungarian honey (Fanni Adrienn Koncz et al. 2018) and recently, Romanian honey (Oroian and Ropciuc 2019). It has been used to detect adulteration in oils (Meenu, Cai, and Xu 2019), beer (Arrieta et al. 2010), Spanish wines (López de Lerma et al. 2013), non-alcoholic beverages (Peres et al. 2009) and milk

(Wei and Wang 2011b) etc. It has scantily been applied for meat and fish evaluations, (Zaukuu et al. 2019), but its potential can be explored several quality control purposes in the meat industry.

In combination with chemometrics and multivariate data analysis, advanced instruments such as the e-tongue and NIRS can be explored for their capabilities to discriminate, detect and predict novel, innovative and undesirable complex forms of food adulteration. Multivariate data analysis in food authenticity are simply, mathematical measures that can be employed to provide real-time and reliable statistical information about a production variation or deviation from quality, otherwise known as fingerprinting.

2. OBJECTIVES

The aim of this thesis was to develop rapid techniques for the determination and prediction of foods with economical and global value using near infrared spectroscopy (NIRS) and electronic tongue (e-tongue). There were primarily two main objectives.

The first main objective was to apply a benchtop spectrophotometer and handheld spectrophotometer to:

1. Develop models that could discriminate, classify and predict different classes of Tokaji wine and also, to classify inferior Tokaji wines that were adulterated with grape must concentrate and sucrose at different sugar concentrations.
2. Develop models that could discriminate, classify and predict whey, beef and pea protein powders that were adulterated with four nitrogen-based adulterants: urea, glycine, taurine and melamine at very low concentrations.
3. Emulate practical situations by testing the feasibility of scanning through a low-density polyethylene (LDPE) plastic bag containing whey, beef and pea protein powders adulterated with urea, glycine, taurine and melamine using a handheld spectrophotometer.

The second main objective was to apply e-tongue to:

1. Develop models that could discriminate, classify and predict different classes of Tokaji wine and also, to classify inferior Tokaji wines that were adulterated with grape must concentrate and sucrose at different sugar concentrations.
2. Determine the optimal dilution level of meat extract for e-tongue evaluation in the meat industry and also to develop standardized sample preparation methods for meat analysis with the e-tongue, using red meat and poultry adulteration as case studies.

3. LITERATURE REVIEW

This section of the thesis reviews existing literature within the scope of the aims defined in the introduction.

3.1. Food fraud

Food is a basic necessity of life. One works hard and earns to satisfy our hunger and relax later but at the end of the day, many of us are not sure of what we eat. We may be eating a dangerous dye, sawdust, soap stone, industrial starch and aluminium foil, which in simple terms may be referred to as adulterants. An adulterant is a chemical substance which should not be contained in food or not above its legal limits (Abhirami and Radha 2015). The addition of adulterants is called adulteration: the addition of ingredients which are not permitted in food (Kartheek et al., 2011). Adulteration is commonly practiced in both branded and unbranded foods in daily life. From the local market to the hyper market, adulteration is prevalent everywhere. Examples of food adulteration include the addition of dye and artificial coloring with cancerous effects to tea leaves and tea powder, addition of corn syrup and sugar to honey could lead to obesity (Kartheek et al. 2011). Other types of adulteration include the addition of pure of diluted ethanol alcoholic beverages (whisky, rum etc.), addition of sweeteners to wine or manipulating the composition of any food item to suit the objectives of the producer at the detriment of the consumer. They are mostly added for business purposes. The European food law stipulates on the "prevention of fraudulent or deceptive practices, the adulteration of food and any other practices which may mislead the consumer" (Jack 2018). Misleading consumers can also be in the form of mislabeling. According to the European Union regulation Number 1169/2011 (European Union 2011), accurate food labelling is of paramount importance and should contain the specific contents of the food being marketed.

The European Union (EU) is a major importer of food and agricultural products, so expected monitoring and controls at its external borders are vital in deterring food adulteration. In contrast, criminals' intent on profiting from food adulteration can be expected to exploit and take advantage of weaknesses in these controls to gain access to the internal market. Subsequently, the EU legal framework on import controls is also heavily reliant upon Member State implementation but neither food adulteration nor compliance with food law generally, has traditionally been a priority concern for Member States (Jack 2018). Also, trends in food production and changes in production systems have compounded with globalization of food supply to make the ultimate supply chain much more complex and easily susceptible to fraud. The most targeted and affected areas of the food production system are meat, liquid and powdered foods. These have been targeted their adulteration is easy to perform can easily go undetected. There have been heavy reports

especially about the adulteration of wine, protein powders and meat. According to the European Commission (European Commission 2018), in 2018 alone, replacement/dilution/addition/removal accounted for 26% of fraud cases in alcoholic beverages whereas 19% of the same criteria was recorded for fraud cases in food supplements. Food fraud and food adulteration in most instances, are dangerous and present health complications to consumers.

3.1.1. Wine fraud and detection techniques

Wine is a beverage produced through the partial or total fermentation of grapes in several steps: harvesting, crushing and pressing, fermentation, clarification, aging and bottling. Wine makers often adopt these different sequential steps but sometimes add variations and deviations along the way to make their wine unique. Each step of the wine production can be an opportunity to perpetuate fraud, therefore tracking wine quality requires a continuous and consistent effort. The wine matrix (composition) is of particular importance within the authenticity testing of foods, because wine production and trade has always been associated with high costs that make it vulnerable to adulteration (Geana et al. 2016).

Wine counterfeiting or adulteration refers to illegal techniques that aim to substitute one valuable component with a cheaper one, to increase profit, thus affecting the final product by changing the wine chemical composition and/or sensorial properties. Wine assessment for traceability and authenticity is a major concern that has rapidly gained a lot of attention. In 2009 alone, more than 1 million adulterated Amarone wine bottles were sold in Italy (Versari et al., 2014). In Asia, anthocyanins are reportedly extracted from black rice (mainly located in the husk) and used as correctors for wine color (Ferrari et al., 2011). On the other hand, anthocyanins have proved to be useful as markers for red wine classification as their presence depends on the grape variety, climate, soil conditions and the winemaking process (da Costa et al. 2018). The origin of white wines from Brazil was confirmed using anthocyanins as markers with the Headspace solid-phase microextraction (HS-SPME) method (Elisa et al. 2013). Wines can also be spiked with controlled amounts of alcohols (methanol or ethanol) between 3-10%v/v, for a deceptive alcoholic content or with other wines of inferior quality but mostly of the same color, to increase the volume (Penza and Cassano 2004). According to the European Commission, wine was the most adulterated food product in 2016 due to the emergence of even more complex adulteration techniques (European Commission 2018). Evaluation of the authenticity of wine involves the assessment of several aspects such as declaration of origin, vintage, variety and production method (Geana et al. 2016). In the last decades, the interest in classifying wines based on their grape variety and geographical origin has increased. The soil, climatic conditions, type of harvest and production conditions contribute to the characteristics that make a wine unique. This concept is linked to the

so-called ’Protected Geographical Status’ defined in European Union law for wines and other food products, which is gradually expanding internationally (da Costa et al. 2018). Information about wine volatile profile may contribute to the achievement of a geographical indication, such as designation of origin, which serves as a benchmark and guarantees product consistency, defining a product that is characteristic of a certain region (Elisa et al. 2013). Currently gaining attention with regards to the concept of wine origin is the Tokaji wine, a botrytized wine of indigenous to the people of Tokaji, a wine producing region in Hungary. Tokaji wine is protected by the European Commission under the ’Protected Geographical Status’ law (EU 2020).

Tokaji wines exist in many specialties and are regarded as a national treasure for their historical significance, unparalleled sensory attributes and organoleptic properties. The wines are produced in the Tokaji region of Hungary, a declared UNESCO World Heritage Site since June 2002 (Makra et al. 2009) for its unique landscape and climate (World Heritage Commitee 2002) that are believed to render some agricultural benefits to the crops in the area. The climate and the grape varieties of Tokaji, contributes to a special process called “noble rot” of the grapes. This involves a partial infection of the berries by the mould *Botrytis cinerea*, combined with physical dehydration due to evaporation that leads to the shriveled state of the berries and makes them desirable for Tokaji wine production. The noble rotten berries have a high sugar content and a unique chemical composition of acids, nitrogenous compounds, polyphenols and aroma compounds that contribute to the wine quality (Magyar & Soos, 2016; Magyar, 2011). Several types of Tokaji wine exist depending on the class (level of shriveling) of the berries used or the method of production. Among common Tokaji wine specialities are: Tokaji Aszu, Tokaji Szamorodni, Tokaji Maslas and Tokaji Forditas. The principle of production for the different specialties is basically a skin contact (maceration) between the botrytized berries and a base wine/grape juice to extract the sugars and aromatic substances (Torino 2016). Extensive details about the compositional quality of different Tokaji wines have been reported by Kerényi (2013) and the effects of noble rotted berries on wine quality have also been detailed by Magyar (2011). The ratio of the botrytized grapes to the extracting wine/juice, the duration of maceration (mostly between 12 - 48 hours) and residual sugar content are all contributing factors in defining the type of specialty being produced (Magyar & Soos, 2016). These factors, with many other parameters, are strictly regulated by law as they strongly influence the selling price of the wines. As of the year 2020, the price of Tokaji wines ranged from about 10 euro/L to about 960 euro/L depending on the residual sugar content (Carl-Gustav 2010). The Tokaji wine Eszencia is considered the sweetest with about 450 g of residual sugar/L (Carl-Gustav 2010) but some vintage bottles can go for as high as 32,855 euro/L (REUTERS 2019). The volumes of production however, are subject to market demand and consumer preference. High market demands often instigates fraudulent

ambitions for financial gains. Addition of natural sweeteners to wine is sometimes accepted when the grapes do not accumulate enough sugars due to inappropriate weather conditions but this remains a relatively controversial topic because the legality of this procedure varies by country, region and even wine type (Regulation (EU) No 1308 2013). For Tokaji wines Hungary, only botrytized berries are permitted, addition of any kinds of sugars, including concentrated juice, is strictly forbidden during the production of Tokaji wine specialties.

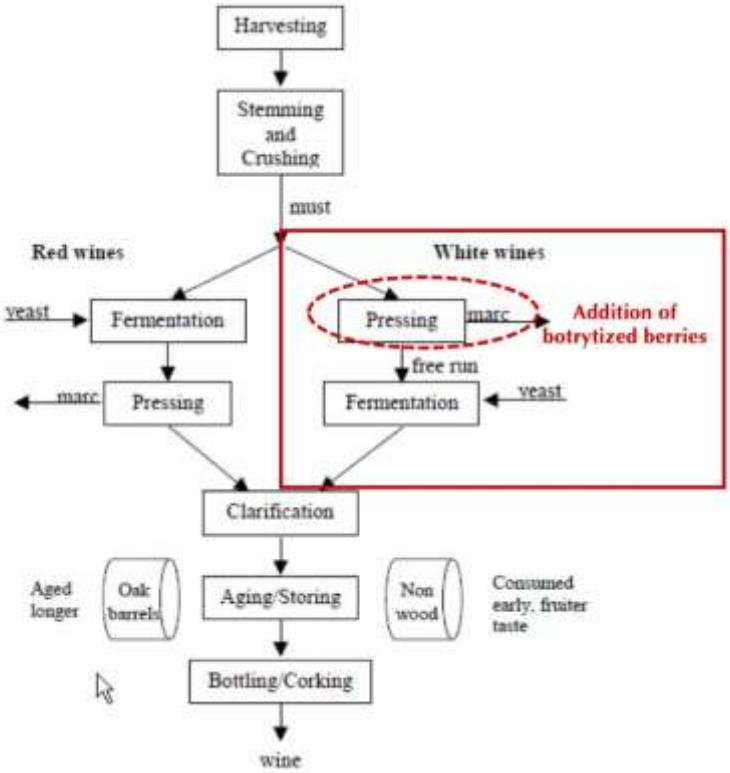

Figure 1: General flow of wine preparation with a unique step for Tokaji wine production
source: (Joshi et al. 2017)

The red rectangle in Figure 1 shows the unique step of preparation for Tokaji wines. Notably, a partial replacement of botrytized berries (noble rotted berries) with grape must concentrate occasionally might be an illegal practice that devalues the wine, puts its reputation at stake, and affects consumer trust in the product. With the wine currently, penetrating the international markets with the prestige of the country on its shoulders, meticulous controls are required to guarantee the quality of the wine. Wine fraud has been tracked with high performance liquid chromatography to authenticate geographical origin of wines with Spanish appellations (Serrano-lourido et al. 2012). Inductively coupled plasma mass spectrometry and optical emission were also used to determine the multielement composition of 272 bottled Slovenian wines (Šelih, Šala, and Drgan 2014). Propylene glycol, sorbic and benzoic acids were also detected as adulterants in semi-dry white wines using headspace solid-phase microextraction (Sagandykova et al. 2017). Descriptive sensory analysis (Vidal et al. 2017) and other advanced tools (Petropoulos

et al. 2017; Kutyła-Olesiuk et al. 2014) have also been used to monitor the quality of different wines but controversial forms of adulteration continue to evolve that require novel techniques of detection. Sugar concentration is a key parameter in wine grading systems. In the case of high sugar concentrations in wine, it is necessary to analyze the sample for the glycerol content as a marker of fermentation depth (Gnilomedova, Anikina, and Gerzhikova 2018). The values of the indicators characteristic of high sugar grapes are typical for the wines obtained by sweetening with grape must concentrate (Cozzolino et al. 2006), which is often measured by the rather expensive methods such as chromatography, nuclear magnetic resonance and mass spectrometry (Jordão, Vilela, and Cosme 2015).

3.1.2. Meat fraud and detection techniques

Meat is a central part of diets around the world and is considered as a primary source of protein across the globe. The world population increased by almost 4 billion in the last 50 years (128%) while the global average meat consumption per capita increased by 75% (Milford et al. 2019). Demand for proteins from plant-based sources has remained stable over time but the same cannot be said for proteins from animal sources. There has been a sharp increase with animal products now accounting for 58% of protein availability per capita/day (Bonnet et al. 2020). This implies that the global meat consumption and production almost quadrupled. Debates about meat production and consumption are often complex and controversial (Wu 2016) but, the same can also be said about meat quality control. Today, animal products provide approximately 30% of the available calories in the European Union (EU) with about 28 g of protein/capita/day, followed by wheat products and dairy products (Bonnet et al. 2020). Controlling meat quality is therefore of paramount importance because meat adulteration or misrepresentation can lead to consumer distrust in the meat value chain which can impact economic revenues. Misrepresentation of meat could equally, also have bad implications from religious and moral perspectives as people have different preferences of meat they wish to consume. Thus, even if different types of meats are mixed and sold in minced forms, their content should be fully labelled in accordance with labelling regulations (European Union 2011).

Primarily, meat from animal sources can be classified into red meat and poultry (McWilliams 2012). In the broad sense red meat, encompasses red fleshed carcass, among which the most patronized are pork, lamb/mutton, beef, and veal (McWilliams 2012). Red meat has been credited with good levels of biological protein, minerals (iron, thiamin, riboflavin, zinc) and vitamins (B6 and B12) (Wyness 2016). They can however, allegedly, contribute to heart diseases depending on their processing (Wu 2016). Poultry meat is the general term used to represent chicken, geese, turkey, duck and other fowls (McWilliams 2012). For reasons such as nutritional

quality and consumer preference or the part of animal that is being sold, the prices all these meat types can vary. For instance, beef is often regarded as a more expensive type of red meat. In the year 2020 in some Hungarian markets (central Europe), beef thigh was sold at a higher price of 3199 HUF/kg (equivalent of 8.88 euro/kg) in comparison to pork thigh which was sold at 1499 HUF/kg (equivalent of 4.16 euro/kg). Turkey breast was also sold at a higher of 1899 HUF/kg (equivalent of 5.27 euro/kg) in comparison to chicken breast which was sold at 1499 HUF/kg (equivalent of 4.02 euro/kg). It is important to note that, some minced meat products already exist in market that can contain a mix of both high-priced and low-priced meat. This is acceptable so long as it is evident in the labelling of the product. What is unacceptable is mixing the high-priced meat with the low-priced meat during mincing and representing it as a 100% version of the expensive one, or falsely stating the applied mixing ratio. This type of adulteration be done at varying concentration levels of meat types depending on the meat type and market demand. Concentration levels with increasing scales of 5% w/w (Alamprese et al. 2013), 10% w/w (Sarno et al. 2020), and 20% w/w (Han et al. 2020) of meat adulteration, are among the most common in literature. The European Union regulation states that the country of origin must be declared in the case of pork and sheep, goat and poultry meat. The previous food law already had these obligations for beef, even to the extent of the countries of animal birth, growth, slaughter and cutting or mixing having to be specified on the label (European Union 2011).

For detecting such adulteration and tampering, many studies have explored different techniques. Common examples are mass spectrometry (Black, Chevallier, and Elliott 2016), polymerase chain reaction (PCR) (Lin et al. 2014; Karabasanavar et al. 2017) and Enzyme-linked immunosorbent assay (ELISA)(Thienes et al. 2019). Due to cost, accuracy, reliability, less sophistication and speed of the analytical process, other methods such as spectroscopy, has become more preferred methods for detecting meat adulteration (Mamani-linares, Gallo, and Alomar 2012; Alamprese et al. 2013, 2016; Z. Zhao et al. 2020; Leng et al. 2020). Another advanced method however, is the e-tongue, but it is generally, suited for liquid samples. As a result, it has been scantly applied in the meat industry although its advantages as an analytical method continuous to be explored in other industrial applications. Few studies have however, adopted the e-tongue to monitor physical–chemical and microbiological changes in pork meat during storage (Gil et al. 2011) and also, the impact of curing agents in meat (Gil et al. 2010). E-tongue could also detect ammonia and putrescine in beef products (Apetrei and Apetrei 2016). Very little was however, mentioned about the sample extraction method in all the studies for easy adaptation of the instrument for meat quality control. In fact, there is no clearly defined sample preparation method for meat analysis with the e-tongue. A standardized sample preparation for e-tongue is necessary

because, a less effective method can decrease the sensitivity of the sensors (Kovacs et al. 2020) which, can negatively affect the results of the instruments.

3.1.3. Protein powder fraud and detection techniques

Proteins are important nutritional requirements with a recommended Dietary Reference Intake (DRI) of 0.8 g of protein/kg of body weight (Wu 2016). In seeking alternative sources of protein for the busy upper echelon consumers, protein supplements have gained attention for their contribution to health. With increasing urbanization, the powdered form has become the most preferred because they are easy to use, store and are easily assimilated upon consumption. In certain contexts, they have even been classified as ergogenic aids mostly sorted by sportive people (Mart et al. 2017) but they can also be expensive depending on the source of their raw materials: beef, pea or whey. Whey protein-based supplements have been promoted as the optimal protein source at maximizing resistance-training outcomes. Beef powder is a new high-quality protein source that reportedly leads to a significant increase in lean body mass performance (Roiffé et al. 2019). Plant-based proteins have less of an anabolic effect than animal proteins due to their lower digestibility, lower essential amino acid content (especially leucine), and deficiency in other essential amino acids but they have recently sorted for their ability preserve skeletal muscle mass to maintain or improve metabolic homeostasis and physical function (Berrazaga et al. 2019).

The purported high price of these products, in addition to an increased demand, has resulted in innovative ideas to manipulate the product for increased profit. Prices of protein powders can range from as low as 10 euro to as high as 100 euro depending on the packaging unit, protein content and protein source (Final Step Marketing 2016). According to the International Society of Sports Nutrition, average serving of protein powders have been reported to be about two servings/day, averagely 42 g/day. Council for Responsible Nutrition (an industry trade group), reported that 11% of adults took protein supplements in 2016 and in America alone, 3.86 billion was spent on protein supplements in the past between 2017 and 2019 years but this amount was still expected rise to nearly 6.57 billion by 2020 (McMillen 2020). Such increasing demands, entices the greed in some producers to explore alternative ways to make more money by manipulating the protein content of some of these products. Common adulterants in protein powder supplements are mainly from cheaper materials and inferior alternatives that have the potential to damage the well-being and health of consumers. Gaining grounds are nitrogen-based substances (Figure 2) that can range from amino acids such as a glycine and taurine to more complex and dangerous chemicals such as urea and melamine which are only acceptable in animal feeds (Tadele and Amha 2015).

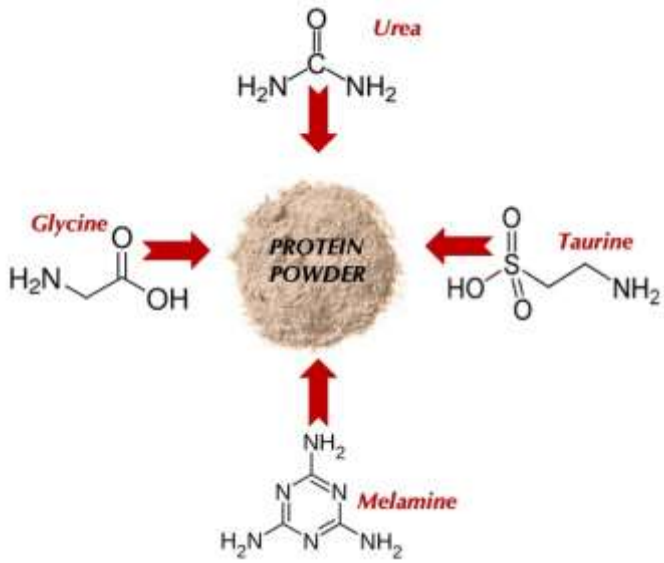

Figure 2: Concept of protein powder adulteration with nitrogenous-based substances: glycine, urea, taurine and melamine

Glycine (H_2NCH_2COOH) is the simplest naturally occurring amino acid that plays crucial roles in protein synthesis (Razak et al. 2017). It is considered as an ideal adulterant for protein powders because it is a naturally sweet nitrogenous based product but at high doses, glycine can cause some negative side effects. Daily endogenous production of glycine is around 125 mg/kg (Mart et al. 2017). Very high intake equivalent to 2.4 g/kg (more than three times the recommended daily allowance) is thought to increase the risk of renal glomerular sclerosis and accelerate osteoporosis (Kohlmeier 2015). Taurine (C_2H7NO_3S) is an important beta amino acid the plays essential roles in many physiological activities. There are no regulations for addition of taurine in protein powders but when used as ingredients in enhanced water beverages, it must not exceed concentrations of 0.0045% (FDA 2015). There is also no clearly defined daily tolerable intake but some reports have indicated allergies such eye and skin irritation and mild diarrhea and constipation (Kohlmeier 2015), adults only need as little as 30–40 mg/day from endogenous synthesis and diet combined. Urea (NH_2CONH_2) is an endogenous product from the degradation of a wide range of nitrogen containing bio-molecules (Hediger et al. 1996). It is generally considered as a toxic product in humans, with approximately 20–35 g being excreted in urine per day (J. Zhao et al. 2018). With appropriate labelling it is accepted as an additive in feed but not in food for human consumption (European Commission 2012). Melamine (C_3H6N_6) is an organic nitrogenous compound synthesized from urea and used in the production of plastics, dyes, fertilizers, and fabrics. Melamine consumption have been linked to nephrolithiasis, chronic kidney inflammation, and bladder carcinoma (Aldrich et al. 2011) but sometimes still ends up in food. A tolerable daily intake of 0.2 mg/kg body weight was adapted by the World Health Organization in

2008 (Tyan et al. 2009). Codex Commission has also adopted a maximum melamine level of 1 mg/kg for powdered infant formula (Codex Alimentarius 2017).

Consequently, the methods and techniques used to authenticate and determine the quality of food products continues to be on the rise. The techniques range from conventional methods such as the Kjeldahl and Dumas crude protein measurement methods to sophisticated ones such as the Bradford and Ninhydrin assays (Field and Field 2010), DNA extraction (Liao et al. 2017), mass spectrometry (Lu et al. 2017), etc. Many of these methods remain effective but the high cost and technicalities associated with their use can sometimes be overwhelming. Also, some mischievous producers have managed to explore the gaps in some of these methods and are using them to their advantage. For instance, Kjeldahl and Dumas crude protein measurement methods are unable to differentiate between nitrogen native to milk and new age nitrogen-rich adulterants with nitrogen in low molecular mass (DeVries et al. 2017). This is because, the methods are based on the assumption that the average nitrogen content of a protein is about 16% (Urbat et al. 2019). An initiative by the Codex Alimentarius committee published that a standard conversion factor of 6.38 rather than 6.25 should be used to determine protein in milk products (Mæhre et al. 2018) but this also translates into extra cost for the industry. In 2008, this meant an additional expense of 88.5million euro affecting the European dairy industry (Koletzko and Shamir 2006). In addition, the conversion factors have their limitations in the fact that, while rich nitrogen-based amino acids such as glycine (18.6% N), histidine (27.1% N), taurine (11.19% N) and melamine (66.6% N), urea (46.62% N) would lower the standard conversion factor, poor nitrogen-based amino acids such as phenylalanine (8.5% N) and tyrosine (7.7% N) (Urbat et al. 2019) would increase it. For instance, urea contains 46% nitrogen; thus each kilogram of urea is equivalent to 2.88 kg of crude milk protein (6.25 × 0.46) (Tadele and Amha 2015). Therefore, substituting rich nitrogen-based adulterants into protein powders is a viable adulteration technique. Practicality of these flaws include melamine poisoning where, the producer diluted the formula by adding melamine, but still passed the protein content test (R. Yang et al. 2009). At least six babies died of kidney failure and tens of thousands became ill (Sharma and Paradakar 2010). There is therefore a need for affordable alternative approaches with low technicalities and the possibility of rapid analysis.

3.2. Emerging methods for food quality control

Food quality assessment is a complex process that translates into assessing all the molecular composition of the food. This can be sometimes overwhelming for the human on biological receptors (sensory evaluation) and the rather expensive and time consuming conventional analytical methods. In the search for optimum food safety, consumers, regulatory bodies and manufacturers are all interested in having reliable analytical tools and information to allow the authentication of foods.

In this regard, near infrared spectroscopy (NIRS) and electronic tongue (e-tongue) have recently gained grounds for their effectiveness in quality control. The NIRS approaches is based on the operating on the interactions of analyte in a defined region of the electromagnetic spectrum of light to give an overview of the compositional structure of the compounds present. E-tongue approach on the other hand, is based on artificial sensors that are able to relay information about the analyte through pattern recognition. Both NIRS and the e-tongue have proven themselves as next generation analytical instruments that will be crucial in the fight against food adulteration. They have been reported to overcome challenges associated with conventional analytical (Figure 3) methods while presenting unique advantages (Table 1).

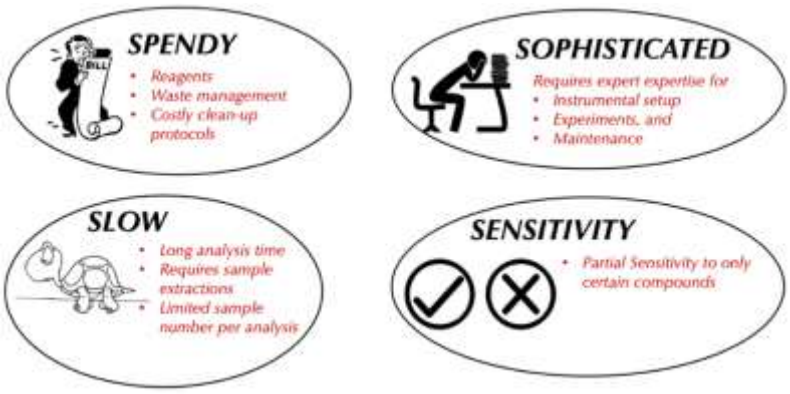

Figure 3: Challenges associated with some common conventional methods

Table 1: Major advantages of the electronic tongue and near infrared spectroscopy

Criterion	E-tongue	Spectroscopy
Rapid	YES	YES
Sophisticated	NO	NO
Requires reagents	NO	NO
Easy to install and use	YES	YES
Good sensitivity	YES	YES
Handheld versions available	YES	YES
Non-invasive	NO	YES
Laborious	NO	NO

3.2.1. Near infrared spectroscopy (NIRS)

NIRS is a well-established technique that operates within a wavelength range of 750 nm (13,300 cm^{-1}) and 2500 nm (4,000 cm^{-1}) (Nicolaï et al. 2007) using a spectrophotometer. Spectrophotometers are instruments composed of a light source, a tool for decomposition of polychromatic light, and a detector (Kagaya and Miyamoto 2017). The NIRS principle basically

encompasses the emission, absorption and reflection of light, which are dependent on the chemical composition of the product (microstructure) and its light scattering properties.

Figure 4 shows main principles of the most commonly used NIR spectrophotometers: scanning grating monochromator, grating polychromator photodiode array spectrophotometers (DA) and Fourier transform NIR analyzers (FT-NIR). The scanning grating spectrophotometers (Figure 4 a) will be primarily used in this thesis.

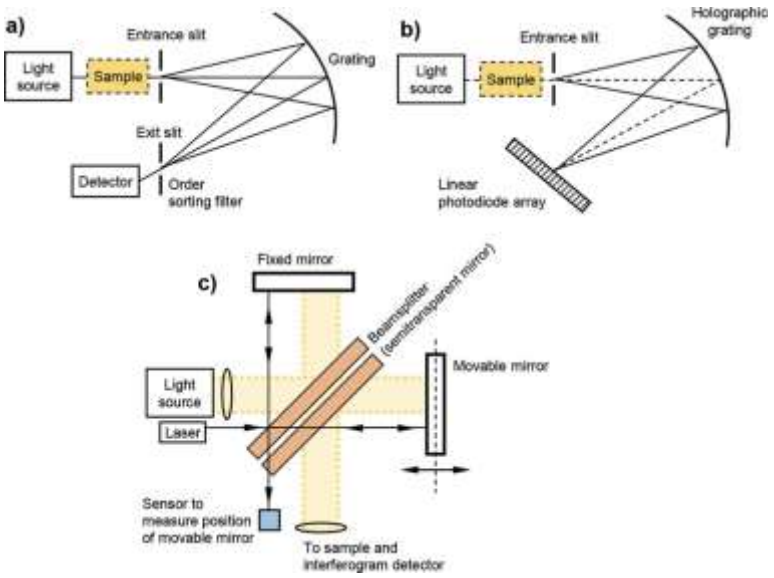

Figure 4: General principles of some common spectrophotometers. Scanning grating monochromator (a) grating polychromator photodiode array (b) Fourier transform (c). Source: (Skvaril, Kyprianidis, and Dahlquist 2017)

In scanning grating spectrophotometers, polychromatic light projects onto diffraction gratings, which then disperses the light into its constituent wavelength. The grating is mechanically rotated so that a narrow group of wavelengths are allowed through the narrow slit (Okazaki 2012). Spectral information can be carried out in different modes as explained in

Figure 5: transmittance, diffuse reflectance and transflectance (Aamer 2011). Diffuse reflectance and transflectance modes will be the focus of NIRS measurements in this thesis.

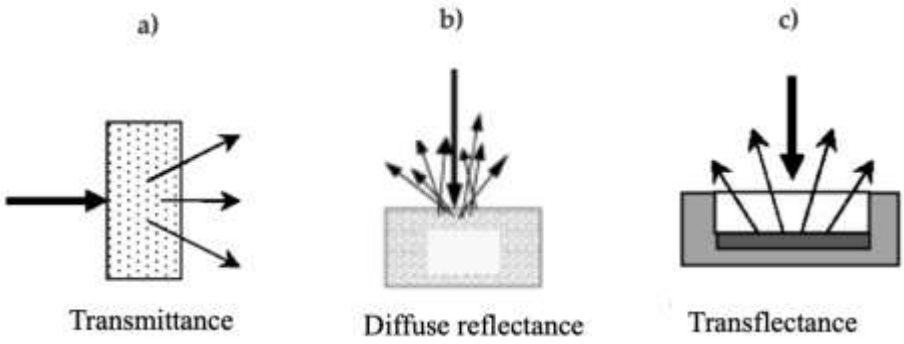

Figure 5: Modes of measurements in NIRS: Transmittance (a), diffuse reflectance (b), transflectance (c). Source: (Aamer 2011)

The most commonly used instrumentation for acquisition of high-quality reflectance spectra of materials moving on a conveyor belt is a fiber-optic coupled head that illuminates the sample usually with halogen light sources (Skvaril, Kyprianidis, and Dahlquist 2017). The most innovative light sources however, are tunable diode lasers, also called super luminescent light-emitting diodes (SLED). Using the semi-conductor technology of diodes, tunable diode lasers are much smaller than the traditional tunable laser, cheaper, have excellent wavelength resolution, brighter, and have lower noise frequencies than tungsten lamps (Agelet and Hurburgh 2010). SLEDs are suitable for measuring weak absorptions at good signal-to noise ratio and as light sources in miniature instruments which will also be explored in this thesis. Improvement of tunable diode lasers allows, controlling emitted light at a specific wavelength, combining light source, and wavelength selection features (Agelet and Hurburgh 2010).

NIRS is routinely employed to give a compositional, functional and sensorial overview of food raw materials, process intermediates and final products. An outstanding advantage of NIRS is that little or no sample preparation is necessary, so the analysis is very rapid (from less than a second to some minutes), free of danger (to both the user and the analyte) and can be carried out on-line (Rateni, Dario, and Cavallo 2017). One of its major strength is the possibility to measure several constituents concurrently and non-invasively. NIRS has been accurately used for geographical identification and classification of Syrah wines from Argentina (Mendoza) and Chile (Central Valley) (da Costa et al., 2018). It was applied for the assessment of metals in Tokaji wine (Murányi and Kovács 2000) where it was concluded that, different production methods of the wine determines the proportion of lead and iron manganese in the wine. In tracking food adulteration, NIRS has been used to detect yellow metanil in tamarind powder (Rukundo et al. 2020), melamine adulteration in milk (Lim et al. 2016) and predict diverse adulterants in cereal products (Ambrose and Cho 2014), herbs and spices (Petrakis and Polissiou 2017), Hungarian honey (Kaszab et al. 2018) and coffee (Dias et al. 2018) etc. It was used for the determination of Sudan I-II-II-IV dye adulteration in spices (Di Anibal *et al.*, 2009), identification of papaya seeds in black pepper (Orrillo et al. 2019), paprika adulteration with lead chromate, 3% w/w lead oxide 5% w/w silicon dioxide, 10% w/w polyvinyl chloride, and 10% w/w gum Arabic (Horn et al. 2018), analysis to detect adulteration in black pepper (Wilde et al. 2019) etc. It can therefore, be inferred from literature that, NIRS is a good tool for qualitative quantitative investigation of foods. In the near infrared region, foods are characterized by certain absorption bands that make it possible to authenticate their quality based on fingerprinting and chemometric or multivariate data evaluations (Lim et al. 2016; Henn et al. 2017; Wold et al. 2020).

3.2.2. Electronic tongue (e-tongue)

Artificial sensors have been widely studied for their potentials in quality assurance. Artificial sensors in engineering can be said to imitate human biological receptors except that, artificial sensors are man-made and can be modified to suit whatever purpose they are desired for. Gaining grounds in the field of sensors are the acoustic, chemical, electrochemical, optical mechanical and thermal sensors etc. (Stroble et al., 2009) but the electrochemical sensors have particularly been lauded for their accuracy, sensitivity and speed when used in analytical instruments such as the electronic tongue.

E-tongue can be defined as an advanced analytical instrument made up of an array of sensors and pattern recognition technologies capable of performing quantitative and qualitative analysis (Winquist 2008). The instrument is composed of an autosampler, an array of artificial sensors, transducer and system unit. The auto sample is designed to contain the sample, the sensor array measures information about the food composition through signals which are processed in the transducer and before storage on the system unit or computer. A full discussion about the different architectures of e-tongue have been reported by Banerjee et al (2016) but the most frequently used are the potentiometric and voltammetric e-tongue (Figure 6 shows their general setup)

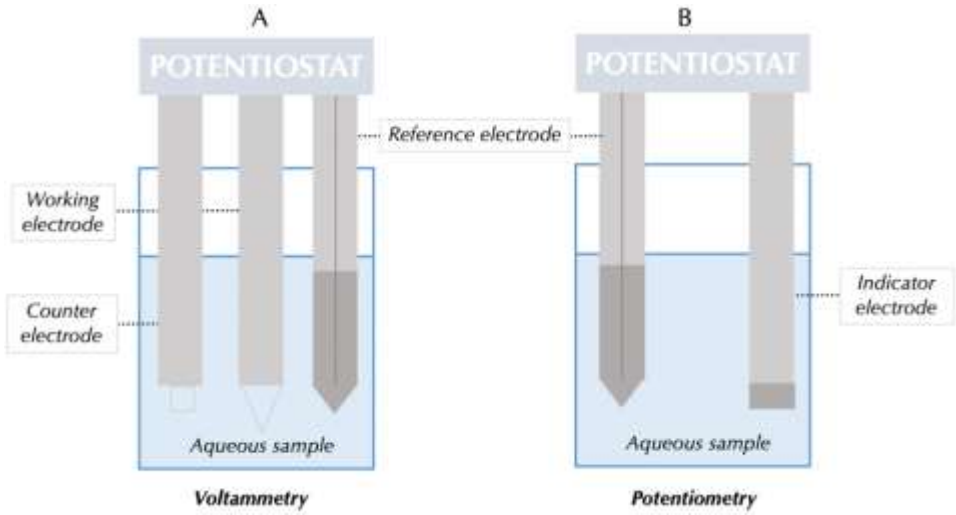

Figure 6: A general setup of a voltammetric e-tongue (A) versus the potentiometric e-tongue (B)

The potentiometric e-tongue operates on the direct application of the Nernst equation and the changes in the electrical potentials of non-polarized electrodes (Magdalena and Wardencki 2015). In the voltammetric e-tongue, the potential is applied on the working electrode, followed by the measurement of the resulting current between the working electrode and the reference electrode. In the development of e-tongue working electrodes or sensors, one of the first class of materials used were lipid membranes in an attempt to mimic the materials of the human tongue (lipid bilayers provide the framework for a cell membrane). Chalcogenide glasses in

electrochemical measurements have also been explored and presented main advantages such as ease of electrode preparation and cross selectivity (Rudnitskaya et al. 2017). Also gaining grounds, are the ion sensitive field effect transistors (ISFET) and chemically modified field-effect-transistor (CHEMFET). These are novel integrated devices in micro electrochemical laboratories. They are very similar to the metal oxide semiconductor field effect transistor (MOSFET) but their sensitive area represents a transistor gate and incorporates the means of transduction from an ion concentration to a voltage (Rita et al., 2017). The e-tongue that would be used in this thesis operated on potentiometry and was adapted to CHEMFET sensors.

Taste is a subjective attribute due to the sensitivity differences in the human tongue of different consumers so advanced instruments such as the e-tongue however, provides reliable non-subjective analysis of taste components in foods as a result of their compositional differences. In food quality assurance, e-tongues have been used for on-site quantification of ethylphenol metabolites (González-Calabuig & del Valle, 2018), storage experiments (Rudnitskaya et al., 2017), quantification of polyphenols (Ce and Ceto 2012) and geographical classification (Lozano et al., 2006) of wines. In food safety, it could detect lead, cadmium, iron in wine (Simões Da Costa, Delgadillo, and Rudnitskaya 2014). With regards to adulteration, it has been used to predict diverse adulterants in tomato paste (Vitalis et al. 2020), honey (Bodor et al. 2017), beer (Polshin et al. 2010), oils (Oroian et al. 2018; Meenu, Cai, and Xu 2019; Apetrei and Apetrei 2014) and coffee (Carpintero et al. 2018). In depth applications of the instruments for food quality have been reported (Bratov, Abramova, and Ipatov 2010; Tian et al. 2018; Zaukuu et al. 2019). The e-tongue has clearly been appraised and acknowledged in food industries for liquid food analysis but its dwindling application for solid food samples such as meat continues to be a challenge that requires attention.

3.3. Data pre-processing techniques and emerging methods

Application of NIRS and e-tongue, always involves more than one variable that can all be influenced by diverse parameters ranging from environmental conditions to sample conditioning. Mathematical correction techniques have therefore been explored to compensate for such drawbacks and are discussed below.

3.3.1. NIRS data pre-preprocessing

Samples that are scanned using diffuse reflectance or transmittance modes, when using spectrophotometers often show significant differences in the spectra. These differences can be due to several conditions such as non-homogeneous distribution of the particles, changes in refractive index, particle size distribution, sample packing/density variability, and sample morphology (surface roughness/shape) (Agelet and Hurburgh 2010). Most often, these conditions are

controlled because they are caused by physical means but even when controlled, they can still contribute to sample pathlength issues that result in additive, multiplicative and wavelength-dependent effects depending on the scanning methodology. Generally, wavelength-dependent scattering can appear as baseline shifts, tilt or a curve scaling difference in some instances, where the effect is more pronounced for longer wavelength region of the spectrum. Spectra measurements of samples from a given chemical matrix that exhibit variable physical characteristics can sometimes look completely different due to these phenomena, masking any subtle chemical variations. Such unwanted variations are unrelated to the chemical response and can be detrimental to the final experiment assessment or yield inaccurate results (Wang and Zhou 2011). It is therefore crucial to apply appropriate pretreatment to minimize such spectra defects for reliable results. Wavelength selection is often the first approach in such situations because it is regarded as the simplest, but most effective pretreatment procedure in spectroscopy, if prior knowledge is available about the regions of interest (Roumiana Tsenkova et al. 2018). Wavelength selection is often performed at the region that are directly related to the analyte of interest and can present advantages such as the elimination of unrelated variables. The scattering effect in NIRS consists of the following (Wehrens 2011): 1) Additive effect, which is reflected as a baseline offset (simple baseline shift) in NIR spectra; 2) Multiplicative effect (pure) that scales the entire spectrum by a given factor, for example due to pathlength differences; 3) Wavelength-dependent baseline variation, where the degree of baseline shift varies with wavelength. In addition to wavelength selection, many types of pretreatment methods have also been reported. Common among these are the derivatives, de-trending (baseline correction), Standard Normal Variate (SNV) and Multiplicative Scatter Correction (MSC) (Bevilacqua et al. 2017). These methods can improve the modeling accuracy depending on the scattering effect in the dataset.

Derivatives are among the most common signal pretreatments applied to spectral data. Derivatives are mainly used to resolve peak overlap (or enhance resolution) and eliminate constant and linear baseline shifts between samples. First and second derivatives are more common in practice than higher-order ones. Spectral derivatives can be calculated by obtaining the differences between two consecutive points, or by smoothing/differentiating specified gap distance; or Savitzky-Golay polynomial fitting (Savitzky and Golay 1964). Some common disadvantages of applying derivatives are noise enhancement and difficult spectral interpretation which are also often dependent on the nature of the data. De-trending is performed through subtraction of a linear or polynomial fit of baseline from the original spectrum to remove tilted baseline variation, usually found in NIR reflectance spectra and Raman spectra with fluorescence background reference (Agelet and Hurburgh 2010). Scaling spectra involves dividing each wavelength data by its standard deviation, which allows each wavelength to have the same weight or relevance during

calibration development. SNV and MSC are two widely known methods that reduce spectral distortions due to scattering. SNV centers and scales each spectrum individually, so each has a mean equal to 0 and standard deviation equal to 1. SNV is often used on spectra where baseline and pathlength changes cause differences between otherwise identical spectra. The method was proposed by (Barnes, Dhanoa, and Lister 1989). MSC correction is achieved by regressing a measured spectrum against a reference spectrum and then correcting the measured spectrum using the slope and intercept of this linear fit (Martens and Naes 1990). This pretreatment method has proven to be effective in minimizing baseline offsets and multiplicative effects. MSC is more complex and memory-consuming than SNV and depends on the whole spectra set, while SNV treats each spectrum individually and independently (Agelet and Hurburgh 2010).

3.3.2. E-tongue data pre-processing

In addressing some of the occurring inaccuracies of e-tongue multidimensional data, several approaches have been adopted, but empirical mathematical logarithms have so far provided better outcomes. Among the first steps in e-tongue data analysis is visual inspection using principal component analysis. This technique helps to detect and eliminate outliers before deciding on what type of signal preprocessing correction is required. Preprocessing is mainly performed to compensate for poor signal-to-noise ratio or otherwise referred to as sensor instability as these can impair e-tongue results. The instability of the chemical sensors is often referred to as sensor drift. It was also defined by Holmberg and Artursson (Holmberg and Artursson 2002) as “a gradual change in any quantitative characteristic that is supposed to remain constant” but in general terms, drift can refer to the inaccurate signal measurements of sensor-based instruments. Drift is a major undesirable characteristic of sensor-based equipment’s which, could occur because of various known and unknown factors such as sensor deterioration due to the evolution of the materials used in sensor development (Holmin et al. 2001). Changes in the environmental conditions of the experiment especially, in temperature or unsatisfactory sensor cleaning can also lead to drift. Even high variation in sample quality or concentration are among the most known effects contributing to sensor drift in the form of memory effect (Kovacs et al. 2020). The response of the sensor depends on what it has recently been exposed to, because remnants of previous samples may be still present on the sensor surface (Holmberg and Artursson 2002). For such reasons, measuring samples with highly different chemical composition and/or concentration may increase the probability of drift in the dataset; this phenomenon is often referred to as the memory effect. The transfer of a portion of the liquid sample on a macro scale due to unsatisfactory sensor cleaning is also a known contributor to changes in sensor signal which, is often called cross-contamination. Another consequence of the cross-contamination would be having some noise that has less to do with the multicomponent response and more to do with the interaction between contaminants and

sensors through a series of adsorption/desorption reactions leading to limited performances (Legin, Kirsanov, and del Valle 2019). These phenomena could be observed for all the different sensors of the e-tongue and could engender the inhibition of electrochemical reactions of interest hence a loss of the electrodes activity (Wei et al. 2017). From a technical and industrial perspective, drift problems hinder the long term operation of all kinds of sensors (Chen and Chan 2008; Polster, Fabian, and Villinger 2009; Owens and Wong 2009). The problem of drift associated stability is an even more important issue for the ion-selective field-effect transistor (ISFET) sensor based electronic tongues. These instruments have high sensitivity, but are often associated with several disturbances (Oelssner et al. 2005). It is therefore of paramount important to always apply some preprocessing technique that can enhance e-tongue signals. Standardized experimental methods, environment conditions and sample dilution before e-tongue analysis have been acknowledged to reduce some of these effect (Szöllosi 2015; Soós et al. 2015) but mathematical corrections have been reported to work best. Many mathematical preprocessing techniques have been developed for e-tongue drift correction (Holmin et al. 2001; Natale, Paolesse, and Legin 2016; Panchuk et al. 2016) devices but three recently developed ones by Kovacs *et al.* (2020), have so far proven to be the most effective and will be used in this study.

3.4. Chemometrics and multivariate data analysis

Both e-tongue and NIRS rely heavily on an arsenal of chemometric and multivariate data analysis tools for rapid real-time evaluation and interpretation of results. Multivariate data analysis and chemometrics, are simply simultaneous observations and analyses of more than one outcome variable in an experiment and can be used to visualize patterns in the data and build classification and prediction models for the dataset. This is contrary to univariate and bivariate analysis which involves just one and two outcomes respectively. Depending on the objectives of the study, the different tools in Figure 7. can be adopted.

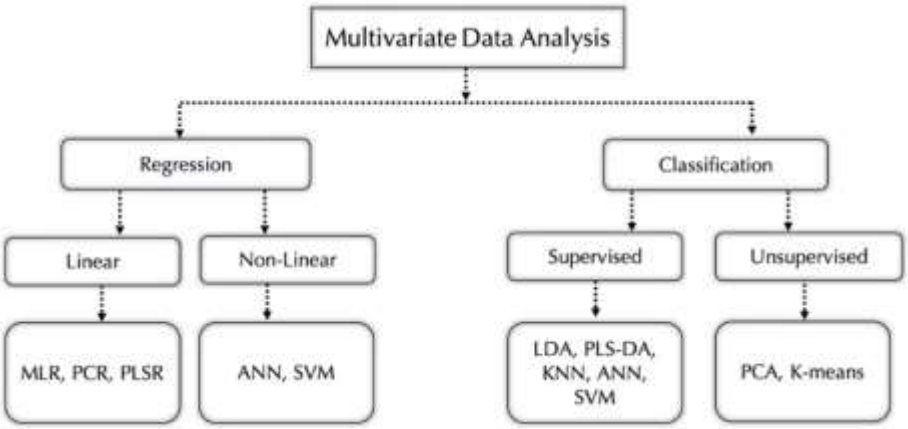

Figure 7: Multivariate data analysis tools commonly used with the e-tongue and NIRS. Source: (Zaukuu et al. 2019)

Adequate data processing is an essential step when using NIRS or biosensor instruments for reliable results. Primarily, Principal component analysis (PCA), linear discriminant analysis (LDA) and partial least squares (PLS) regression will be used in this thesis.

PCA is most known for data visualization purposes. It is favorable because it is a way of identifying patterns in data and it expresses the data in such a way that it highlights their differences and similarities. It is an outlier sensitive statistical method that reduces the amount of data to a smaller number of newly derived variables which represent the original data adequately (Shlens, 2005). Detailed concepts of PCA application have been reported by Joliffe & Morgan (1992).

LDA is most known as a classification technique. It is often used for multi-class classification of different samples. It is a supervised method so the class membership has to be known for the analysis (Granato et al., 2018). Results are often expressed as classification accuracies in terms of recognition (calibration) or validation (prediction) that can be used to extract the required information from the usually convoluted spectra in NIRS (Hu et al. 2019) or the e-tongue sensor signals. Target accuracies are often dependent on the objective of the study and analytes being studied but for detecting food adulteration, the ratio between within-class and between-class variances should be maximum in a lower dimensional space because some adulterants can present severe health complications.

PLS, seeks to maximize the covariance between the X and Y blocks, in a way that the new latent variables not only explain the variability of X but are also maximally correlated to Y (Bevilacqua et al. 2017). Correlation is the measure of the degree of association between two variables when both are measured on a series of objects. The strength of the correlation is given by the coefficient of determination (R^2). R^2 or adjusted regression coefficient (R2adj) close to one is recommended in literature (Schunn and Wallach 2005). R^2adj adjusts for the adjusted number of explanatory terms in a model relative to the number of data points and its value is usually less than or equal to that of R^2. When comparing models, the one with the highest adjusted coefficient is the best model (Granato, de Araújo Calado and Jarvis, 2014). However, R^2adj alone is not enough to evaluate goodness of fit as, the calibration error, i.e., the residuals of the calibration data need to be considered. This is often referred to as Root Mean Squared Error Calibration (RMSEC) or RMSECV when cross-validation is used (Kamiloglu 2019). Depending on the sample size, an RMSEC or RMSECV close to zero is often recommended (Simões Da Costa, Delgadillo, and Rudnitskaya 2014). Details about PLS regression methods have been reported by Palit et al. (2010).

3.5. Limit of detection

The goal of analytical procedures is generally to achieve qualitative and/or quantitative result with acceptable uncertainty levels. Therefore, theoretically speaking, “validation ” really indicates “measuring uncertainty” (Sengul 2016). In practice, method validations are performed by evaluating a series of method-performance characteristic such as limit of detection minimum value (LODmin), limit of detection maximum value (LODmax), limit of quantification minimum value (LOQmin), limit of quantification maximum value (LOQmax), explained variance X (the actual dataset) and explained variance Y (the predicted dataset). There have been some lack of agreement among researchers as to the terminology that is best suited to describe some of these parameters (Armbruster and Pry 2008) but in statistics, explained variation measures the proportion to which a mathematical model accounts for the variation of a given data set (Henn et al. 2017). LOD can be defined as the lowest amount of analyte in the sample, which can be detected but not necessarily quantitated under stated experimental conditions. LOQ is generally defined as a parameter of quantitative assays for low levels of compounds in sample matrices (Shrivastava and Gupta 2011). Typically, LOQ will be found at the same or higher concentration than LOD, but how much higher depends on the specifications for bias and imprecision used to define it (Armbruster and Pry 2008). These terminologies are often used to describe the smallest concentration of an analyte that can be reliably measured by an analytical procedure. Lower and upper limits of the LODminimum and LODmaximum (LODmin/max) correspond to the calibration samples with the lowest and largest extrapolated leverages to zero analyte concentration (Lukacs *et al.*, 2018). According to the International Union of Pure and Applied Chemistry (IUPAC), LODmin/max measurements are recommended because they bring together two important analytical concepts: the sensitivity and the precision in the analytical determinations (Allegrini and Olivieri 2014). In spectroscopy, these can be used to assess the measurement performance directly related to the experimental conditions (Henn et al. 2017). Several approaches can be used to calculate detection and quantification limits through visual evaluation, signal-to-noise, standard deviation of the blank, and calibration curve methods (Sengul 2016) but the calibration curve methods will be adopted in this thesis because this method yields more homogeneous distribution that can result in a more relevant mathematical assessment (Shrivastava and Gupta 2011). LODmin/max, LOQmin/max and explained variance will be used to evaluate models built to predict protein powder adulteration in this thesis.

4. MATERIALS AND METHODS

This section of the thesis focuses on how all the experiments were performed to achieve the set objectives. For easy reading and understanding, it has been divided into three main parts: sample preparation, applied methods and statistical methods that will all focus on the foods under investigation.

4.1. Sample preparation

The sample preparation section focuses on how all the samples in each experiment were acquired and prepared before instrumental analysis with the applied methods to achieve the set aim and objectives of the thesis.

4.1.1. Determination of Tokaji Aszu and Tokaji Forditas wine adulteration

Grape must concentrate (G.M.C) and four Tokaji wines of different quality grade (in increasing order of quality): Tokaji Forditas II, Tokaji Forditas I, Tokaji Aszu II and Tokaji Aszu I, were obtained from experts at the winemaking Tokaji region of Hungary (Figure 8).

Figure 8: Authentic Tokaji wines from the Tokaji region of Hungary acquired as raw materials for the determination of Tokaji wine adulteration

The wines were produced according to the standard requirements of Tokaji wine production as described: maceration of first class botrytized berries with base wine (fermented grape juice) to get Tokaji Aszu I wine and maceration of second class botrytized berries with base wine to get Tokaji Aszu II wine. Tokaji Forditas I and Tokaji Forditas II wines were prepared by reusing the botrytized berries from Tokaji Aszu I and Aszu II wine production for a second maceration with base wine, which is the normal practice in making Tokaji Forditas wines. The wines had a maceration period of 48 hours and resulted in diverse sugar concentrations that were determined by HPLC at the Department of Oenology, Szent Istvan University (now, Hungarian University of Agriculture and Life Sciences).

Tokaji Forditas I and Forditas II wine wines were manipulated with G.M.C of 775.3 g/L sugar concentration to mimic the sugar concentrations of the authentic Tokaji wines. The adulteration was done in steps, resulting in four different adulteration levels (C1 -C4) each for Forditas I wine and Forditas II wine adulteration (Figure 9).

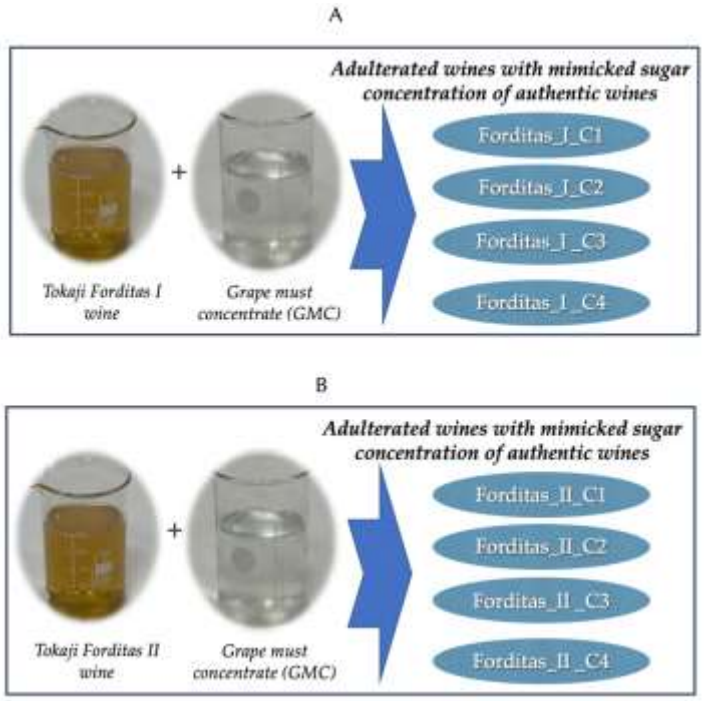

Figure 9: General setup of sample preparation for Tokaji Forditas I wine adulteration (A) and Tokaji Forditas II wine adulteration

The sugar concentration of the adulterated wines covered the range of sugar concentration generally existing in the authentic Tokaji wines). The purpose of this artificial adulteration was to monitor the possibility of producing wines of similar quality to the authentic Tokaji wines by sugar manipulation. Also included was a complex wine referred to as “Base_sugar” in this study. This wine contained sucrose during its refermentation and was not macerated with botrytized berries, but was refermented after the addition of sugar. It was meant to represent complex forms of adulteration. Three repeats of each sample was prepared using a 50% dilution factor as this was reported to be the recommended dilution level for wine analysis with the electronic tongue (Soós et al. 2015).

Table 2: List of samples showing the non-adulterated Tokaji wine, adulterated Tokaji wine and their corresponding sugar concentrations

Sample number	Tokaji wines	Adulterated Tokaji wines	Sugar concentrations g/L
1	Forditas_II		98.9
2	Forditas_I		130.2
3		Forditas_II_C1	130.2
4	Aszu_II		168.2
5		Forditas_II_C2	168.2
6		Forditas_I_C1	168.2
7		Forditas_I_C2	203.5[a]
8		Base_Sugar	238.8
9		Forditas_II_C3	238.8
10		Forditas_I_C3	239.6[b]
11	Aszu_I		254.5
12		Forditas_II_C4	254.5
13		Forditas_I_C4	254.5

[a]: the value is derived from (Forditas_I + Base_Sugar)/2

[b]: the value is derived from (Aszu_I + Base Sugar)/2

4.1.2. Determination of optimal dilution and optimal extraction for meat analysis with the e-tongue

Fresh poultry (turkey and chicken breast) and red meat (beef and pork thigh) were purchased from reputable supermarkets (SPAR) in Budapest, Hungary and transported to the laboratory for processing and analysis. One kilogram of each meat type was minced in the laboratory with a commercially made mincer before commencing the experiments.

4.1.2.1. Determination of optimal dilution

Samples are also often recommended to be diluted before e-tongue analysis as a means of prolonging the long-term usage of the sensors and also, to achieve a range where sensor sensitivities are optimal. Three dilution levels were tested to identify the one that gave the best accuracies for detecting meat adulteration. Figure 10 shows the basic steps that were taken to achieve this objective.

Figure 10: Flow chart for the determination of optimal meat extraction.

Minced turkey and chicken were artificially adulterated to four different concentration levels: 100% 97%, 95% and 90% w/w of turkey/chicken to have a total of 20 g per sample (meat mixture concentration) as shown in Table 3. The concentration levels for this experiment were determined based on the commonly reported ranges in literature (Alamprese et al. 2013; Sarno et al. 2020; Han et al. 2020) but the extra lower level of 97% w/w turkey was included to study the feasibility of e-tongue in discriminating lower concentrations than those reported in literature.

Table 3: Mixture combination for turkey and chicken adulteration for determination of optimal dilution

sample ID	Turkey (% w/w)	Chicken (% w/w)	Turkey (g)	Chicken (g)	% w/v of meat mixture
05_100	100	0	20.00	0.00	0.50
05_097	97	3	19.40	0.60	0.50
05_095	95	5	19.00	1.00	0.50
05_090	90	10	18.00	2.00	0.50
10_100	100	0	20.00	0.00	1.00
10_097	97	3	19.40	0.60	1.00
10_095	95	5	19.00	1.00	1.00
10_090	90	10	18.00	2.00	1.00
20_100	100	0	20.00	0.00	2.00
20_097	97	3	19.40	0.60	2.00
20_095	95	5	19.00	1.00	2.00
20_090	90	10	18.00	2.00	2.00

Each meat mixture was extracted by transferring the sample into a 200 mL volumetric flask and filled up to volume with distilled water then homogenized by vigorously shaking in the flask for 3 minutes. It was then filtered using a wire mesh filter (1 mm pore size) to obtain the stock filtrate. This method was referred to as the “Raw meat extraction method”.

From the stock filtrate of each of the prepared meat mixtures, 0.5%, 1% and 2% w/v dilutions were prepared by pipetting 5 mL, 10 mL and 20 mL respectively, into separate 100 mL volumetric flasks and homogenized by shaking the flask for 3 minutes before filling up to volume with distilled water, then transferred into 100 mL glass beakers for e-tongue analysis.

4.1.2.2. Determination of optimal extraction method

Overall extraction efficiency often depends on the total extractable material weight or the individual components after extraction, this has been reported to be most significantly affected by the extraction temperature (Liu, Ang, and Springer 2000). Based on this, three different extraction methods were developed that fell within the sustainable green technology category of the Sustainable Development Goals Index (SGDI) (Guo et al. 2020), non-chemical related analysis for environmental sustainability. They were "raw meat extraction method", "frozen meat extraction method" and "cooked meat extraction method".

Raw meat extraction method (previously used for the determination of optimal dilution): 20 g of meat mixture was extracted as described in the experiment for the determination of optimal dilution to obtain the stock filtrates and as shown in Figure 11. From the stock filtrate, the pre-determined optimal dilution level was pipetted into a 100 mL volumetric flask, filled up to volume with distilled water, homogenized and transferred into 100 mL glass beakers for e-tongue analysis. This method was developed to test the possibility of extracting meat components using distilled water at room temperature (25 °C).

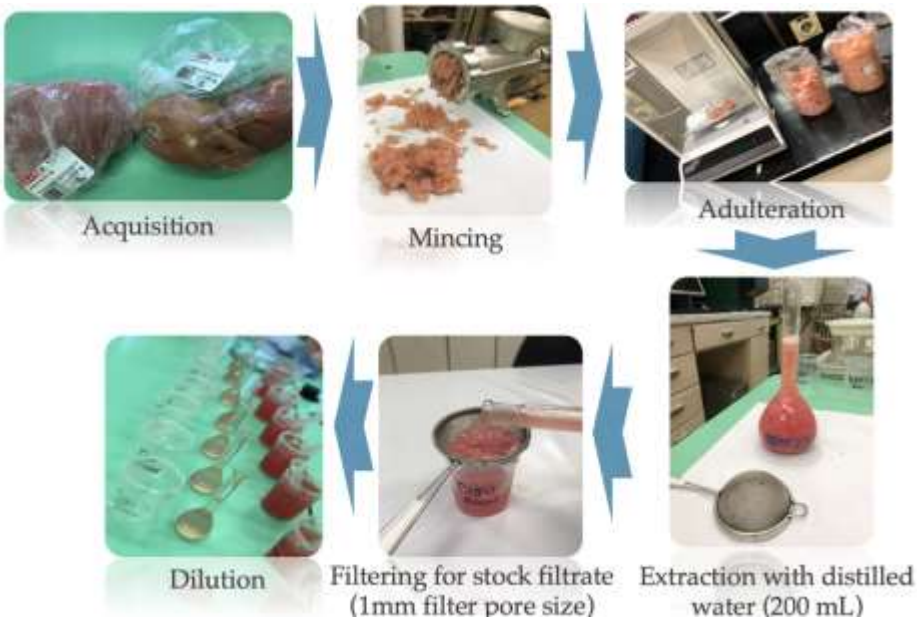

Figure 11: Sample preparation for raw meat extraction with distilled water

Frozen meat extraction method: 20 g of meat mixture was stored by freezing at a temperature -18 °C. The Frozen samples were removed on the second day of storage and put into a water bath of 50 °C for 20 minutes for them to thaw. Chief determinants of thawing are time temperature and the size of the cut, the higher the thawing temperature, the shorter the time required (Belle et al. 1952), that is why the meat samples were put in the water bath of 50 °C for 20 minutes. After thawing, the samples were then prepared in a similar way to the raw meat

extraction method (Figure 12). The method parameters were decided on the basis that, thawing weight loss of meat principally consists of "drip" (the fluid that oozes from the meat) (Aidani et al. 2014). The feasibility of the e-tongue to discriminate the different meat mixtures based on meat components from drip was the primary interest.

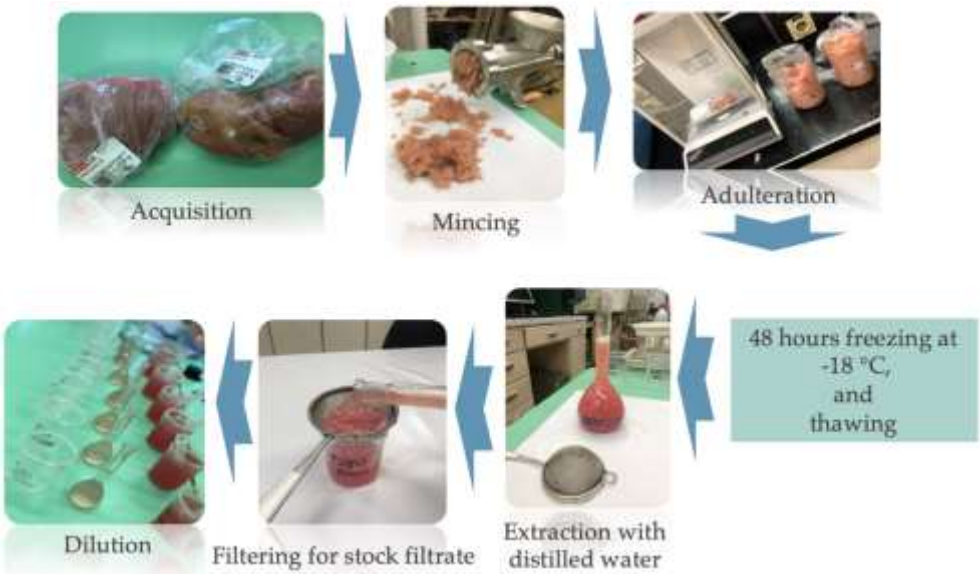

Figure 12: Sample preparation for meat extraction by freezing

Cooked meat extraction method: 20 g of meat mixture was put in a cooking pot containing 200 mL distilled water at room temperature and covered with the lid before boiling for five minutes with a commercial electric hot plate (Sencor, SCP 1500). It was afterwards, filtered with a wire mesh filter (1 mm) to obtain the stock filtrate. From the stock filtrate, the pre-determined optimal dilution level was pipetted into a 100 mL volumetric flask, filled up to volume with distilled water, homogenized and transferred into 100 mL glass beakers for e-tongue analysis. Figure 13 shows the process flow for this method.

Figure 13: Sample preparation for meat extraction by cooking

Six defined concentrations of poultry (Table 4) and red meat (Table 5) mixtures were used to evaluate all the three described sample preparation methods. The efficiency of the three developed mixtures was determined by their ability to discriminate the different meat mixtures after e-tongue analysis with diluted extracts prepared from the three methods. Three repeats were prepared for each meat mixture concentration level resulting in a total of 18 samples for poultry and red meat adulterated mixture respectively, for each of the three sample extraction methods.

Table 4: Mixture combination for turkey and chicken adulteration determination of optimal extraction

sample ID	Turkey (%)	Chicken (%)	Turkey (g)	Chicken (g)
T100	100	0	20.00	0.00
T099	99	1	19.80	0.20
T097	97	3	19.40	0.60
T095	95	5	19.00	1.00
T090	90	10	18.00	2.00
T080	80	20	16.00	4.00

Table 5: Mixture combination for beef and pork adulteration determination of optimal extraction

sample ID	Beef (%)	Pork (%)	Beef (g)	Pork (g)
B100	100	0	20.00	0.00
B099	99	1	19.80	0.20
B097	97	3	19.40	0.60
B095	95	5	19.00	1.00
B090	90	10	18.00	2.00
B080	80	20	16.00	4.00

4.1.3. Determination of whey, beef and pea protein powder adulteration

Whey protein powder, beef protein powder, pea protein powder, taurine, and glycine were provided by SCITEC Ltd. (Dunakeszi, Hungary). Urea was acquired from Elemental SRL (Bihor, Romania) and melamine was acquired from Carl Roth GmbH (Karlsruhe, Germany). All three protein powders were artificially adulterated using urea (U), glycine (G), taurine (T) and melamine (M) as adulterants. The nitrogen content (N) of the adulterants were: urea (46.62% N), glycine (18.65% N), taurine (11.19% N) and melamine (66.60% N). To have equal particle size, protein powders and adulterants were sieved through a wire mesh sieve with pore size (0.6mm) before adulteration.

A combination pattern was developed to contain single adulterant mixtures (U, G, T, M), dual mixtures (GT, UG, GM, UT, TM, UM) and multiple mixtures (UGT, GTM, UGM, UTM, UGTM). This resulted in a total of 15 different mixture combinations. All the mixture combinations were prepared to have total adulterant concentrations of 0.5%, 1%, 1.5%, 2%, 2.5%,

and 3% w/w in whey, beef and pea protein powders. The exact amount of protein powder used in each mixture was calculated based on the nitrogen content of the individual adulterants using melamine as the base adulterant because it had the highest nitrogen content of 66.6%. Triplicates of each mixture were prepared with each weighing three gram (total mass after adulteration) and rigorously homogenized by shaking for 3 minutes. In total, there were 273 samples per protein type. Barcode system was used for easy labelling and identification of the samples during scanning with the instruments.

4.2. Applied methods

This section covers all the technical details about the NIRS and e-tongue instruments used for the experiments, the brands, modus operandi and how they were applied in each experiment to achieve the set aim and objectives of the thesis.

4.2.1. Near infrared spectroscopy (NIRS)

The MetriNIR (MetriNIR Research, Development and Service Co., Budapest, Hungary) with a wavelength range of 750-1700 nm and a spectral stepping of 2 nm was used as the benchtop spectrophotometer (Figure 14 A). The NIR-S-G1 (InnoSpectra Co., Hsinchu, Taiwan) with a wavelength range of 900-1700 nm and a spectral stepping of 3-4 nm was used as the handheld spectrophotometer (Figure 14 B).

Figure 14: MetriNIR benchtop spectrophotometer (A) and NIR-S-G1handheld spectrophotometer (B)

4.2.1.1. Spectra acquisition of Tokaji wine and protein powder mixtures with NIRS

For determination of Tokaji wine adulteration, three consecutive transflectance spectra were collected for each repeat sample using benchtop and handheld spectrophotometers with an optical glass cuvette of 0.4 mm layer thickness. There was a total of 117 spectra per spectrophotometer representing three consecutive scans for each of the four authentic wines and their three repeats (12 samples), adulterated Forditas I wine and their three repeats (12 samples),

adulterated Forditas II wine and their three repeats (12 samples) and base sugar wine and its three repeats (3 samples).

For determination of protein powder adulteration, three consecutive diffuse reflectance spectra of each sample repeat were collected using three setups: benchtop spectrophotometer with optical glass cuvette (Figure 15 A), handheld spectrophotometer with optical glass cuvette of 0.4 mm layer thickness (Figure 15 B), and handheld spectrophotometer with low density polyethylene (LDPE) zip lock bags (Figure 15 C). There was a total of 2457 spectra per setup representing three consecutive spectra for each of the whey (273 samples), beef (273 samples) and pea (273 samples) protein powder mixtures. For the scans taken with the optical glass cuvette, each consecutive scan was taken after rotating the cuvette in the spectrophotometer. There was no stirring or re-packing between consecutive scans of the protein powder mixtures and no backing material was placed behind the sample. For the scans taken with the LDPE plastic bag, each consecutive scan was taken when all three gram of each protein powder mixture was moved to the tip of the plastic bag as much as possible (Figure 15 C) Layer thickness of the LDPE plastic was 0.09 mm and thickness after filing in with powdered samples was 5-6 mm.

All spectral acquisition was performed at room temperature, the temperature of the room was monitored using the Voltcraft DL-121TH Multi-Data logger to reveal any substantial changes in temperature and relative humidity. The "aquap2" package (Kovacs and Pollner 2016) in R-project was used for spectral analysis.

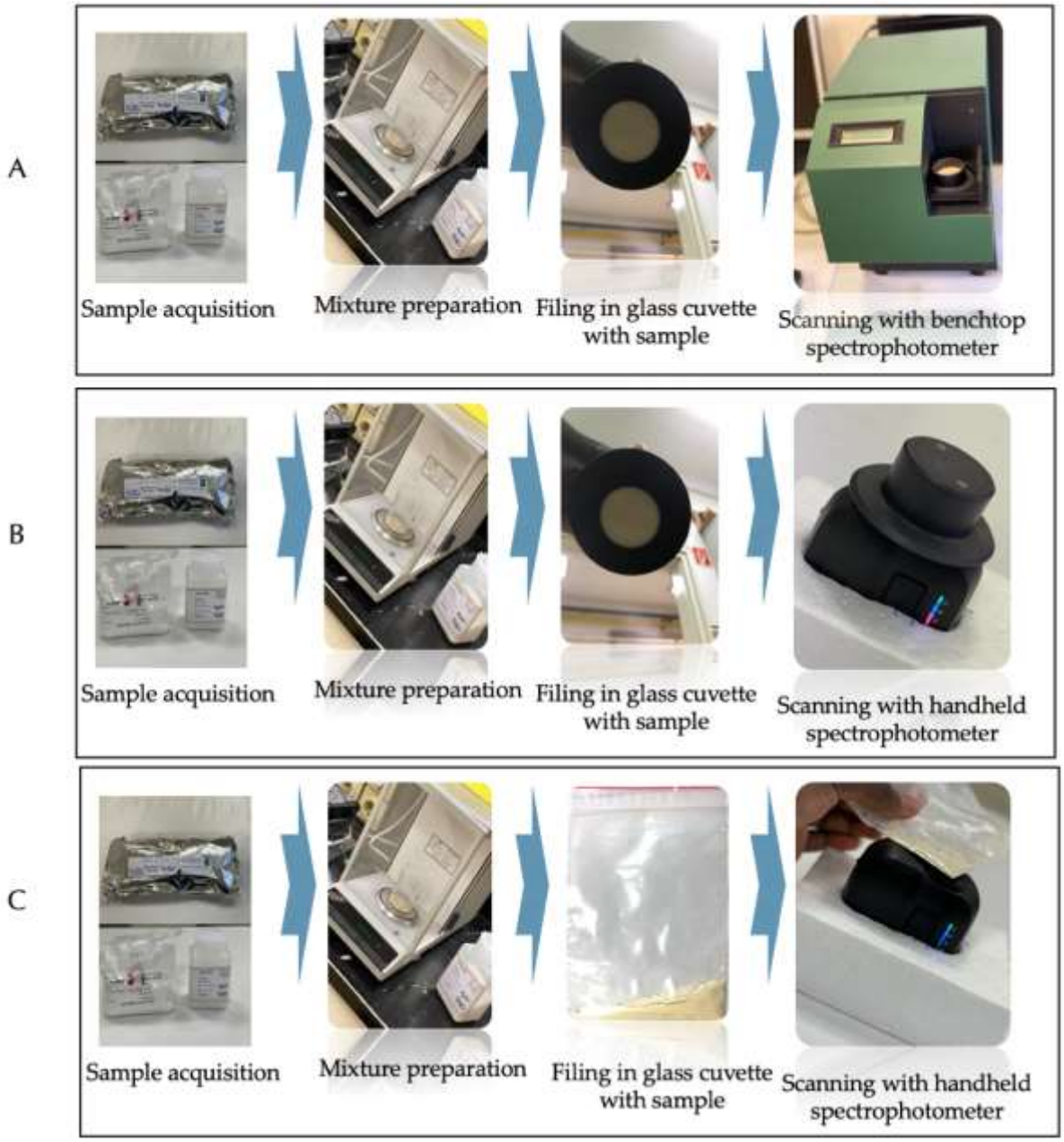

Figure 15: Methods for spectral acquisition of adulterated protein powder using the bench top and handheld instrument through a glass cuvette and plastic surface

4.2.2. Electronic tongue (e-tongue)

The Alpha Astree potentiometric e-tongue (Figure 16) with Ag/AgCl reference electrode and Chemical Modified Field Effect Transistor (CHEMFET) sensors (for food applications) from Alpha M.O.S (Toulouse, France) was used to discriminate the different wine and meat mixtures based on pattern recognition. According to the manufacturer's recommendations, the instrument was conditioned in two phases: with 0.01 N HCL and with mixture of the samples under study, before the analysis to reduce sensor drift and memory effect (Alpha Astree 2010).

Figure 16: The Alpha Astree potentiometric e-tongue by AlphaM.O.S (Toulouse, France)
Source: (Alpha Astree 2010)

4.2.2.1. Calibration and signal acquisition for Tokaji wine and meat mixtures with e-tongue

Aliquots of the different wine mixtures after 50% aqua dilution were mixed to obtain a total of 100 mL solution that was used to calibrate the instrument before signal acquisition. For signal acquisition, there was a total of 156 data points representing four measurements for each of the four authentic wines and their three repeats (12 samples), adulterated Forditas I wine and their three repeats (12 samples), adulterated Forditas II wine their three repeats (12 samples) and base sugar wine and its three repeats (3 samples).

Aliquots of pure turkey meat extract after tenfold aqua dilution was used to calibrate the instrument before signal acquisition for the determination of optimal dilution. For signal acquisition for the determination of optimal dilution, there was a total of 48 data points representing four measurements of each of the four different meat mixtures (100%, 97%, 95% and 90% w/w turkey/chicken) prepared using 0.5% w/v, 1% w/v and 2% w/v dilutions.

Aliquots of pure turkey meat extract and pure beef extract after 10x aqua dilution were respectively, used to calibrate the instrument for the determination of optimal extraction method poultry meat mixtures and red meat mixtures. For signal acquisition, there was a total of 216 data points representing four e-tongue measurements of the six different meat mixture concentrations of turkey/chicken (100%, 99%, 97%, 95%, 90% and 80% w/w) prepared in three repeats and extracted using each the raw meat extraction, frozen meat and cooked meat extraction methods, separately.

During the e-tongue measurements for determination of Tokaji wine adulteration, determination of optimal dilution for meat analysis and optimal extraction for meat analysis, the

tested sample volume was 100 mL, the sampling time was 120 seconds, the sampling frequency was 1 second, and the cleaning time with distilled water was 20 seconds. The last 10 seconds of the sensor signals, representing stabilized and optimal sensitivity of the different sensors were exported for statistical evaluations in R-project.

4.3. Statistical methods

The purpose of statistical methods when using NIRS and e-tongue, is to calibrate the spectra or signal responses and permit fitting of the multidimensional output (Wei et al., 2017). This section of the materials and methods covers all the details about how the different preprocessing methods (pretreatments). Chemometric analysis was applied on the datasets from all the different experiments.

4.3.1. Spectra preprocessing for Tokaji wine and protein powder experiments with NIRS

Raw spectra inspection was performed to obtain the best wavelength selection range for the dataset from the Tokaj wine and protein powder experiments. Peaks and wavelengths that showed some correlations with the composition of the different analytes were also identified and discussed. Outlier detection was performed in principal component analysis (PCA) before applying preprocessing techniques. Different types of pretreatment and their different combinations were tested on the raw spectra for their ability to reduce baseline shifts, spectral noise, correct additive and/or multiplicative effects in spectral data and enhance spectral information. The pretreatments were selected based on their frequent use in literature for liquid and powdered samples and included Savitzky-Golay smoothing filter (smoothing point 21), 1st derivative Savitzky-Golay smoothing filter (smoothing point 21), 2nd derivative Savitzky-Golay smoothing filter (smoothing point 21), Standard Normal Variate (SNV), Multiplicative Scatter Correction (MSC) and detrend. Their tested combinations were Savitzky-Golay (smoothing point 21) + MSC, Savitzky-Golay (smoothing point 21) + SNV, 1st derivative Savitzky-Golay (smoothing point 21) + MSC, 2nd derivative Savitzky-Golay (smoothing point 21) + MSC. This was performed on the dataset from both the Tokaji wine and protein powder experiments.

For the dataset from the Tokaji wine experiment, the pretreatment that could classify all the authentic and adulterated wine mixtures with the highest average recognition and prediction (cross-validation) accuracy was deemed the best and was applied before developing detailed classification models. This was done using the dataset using the dataset from both the benchtop and handheld spectrophotometers.

For the dataset from the protein powder experiment, the pretreatment that could classify all the protein powder mixtures with the highest average recognition and prediction (cross-validation) accuracy regardless of the protein source or adulterant concentration, was deemed the best and

was applied before developing detailed classification models. The pretreatment that could predict melamine concentration in the whole dataset with the highest R^2CV and lowest RMSECV regardless of protein source or mixture combination, was deemed the best and was also applied before building detailed regression models. This was done using the dataset from the three setups: benchtop spectrophotometer with optical glass cuvette, handheld spectrophotometer with optical glass cuvette and handheld spectrophotometer with LDPE.

4.3.2. E-tongue signal correction for Tokaji wine and meat experiments

Sensor signals for the dataset for the determination of Tokaji wine adulteration, determination of optimal dilution and determination of optimal extract were all pretreated with the additive correction relative to all samples (Kovacs et al. 2020). This mathematical drift correction method is appropriate in cases where, measurements are performed with the same sample set and sequence. The key to this method lies primarily in the simplicity of its calculation and secondarily that, there is no assumption about the behavior of the drift so it can correct the effect of varying temperature or memory.

In addition to the additive correction relative to all samples, a sensor optimization process was performed for the dataset from the determination of optimal extraction. This was necessary because of the poor results achieved from pre-analysis even after the additive correction relative to all samples was performed. The sensor optimization process was performed to improve both LDA and regression results. To improve the LDA results, sensor optimization was done by running LDA simulation models in six steps. One sensor was removed in each step of the simulation and the average values of cross-validation were compared to the average value of cross-validation when all seven sensors were used. The sensor combination that produced the highest accuracy after cross-validation was selected and used to develop the subsequent LDA models. The same thing was done to improve the accuracies of regression models too but this time, using the RMSECV's as the parameter of evaluation. The sensor combination that produced the lowest RMSECV was selected and used to develop the subsequent regression models.

4.3.3. LDA multi-class classifications for determination of wine, meat and protein powder adulteration

For determination of Tokaji wine adulteration, classification models were firstly, developed to classify authentic wines using dataset from e-tongue, benchtop spectrophotometer and handheld spectrophotometer. Models were also developed to classify authentic wines, Forditas I and Forditas II adulterated wine mixtures using e-tongue, benchtop spectrophotometer and handheld spectrophotometer.

For determination of optimal dilution for meat analysis with e-tongue, classification models were developed to classify 100%, 97%, 95% and 90% w/w turkey/chicken after 0.5%, 1% and 2% w/v dilution. The dilution level that gave the highest average recognition and prediction (cross-validation) accuracy was considered to be the optimum dilution level for meat analysis with e-tongue.

For determination of optimal extraction method for meat analysis with e-tongue, classification models were developed to classify 100%, 99%, 97%, 95%, 90%, 80% w/w turkey/chicken and beef/pork respectively after extraction using raw meat, frozen meat and cooked extraction method and dilution with the determined optimal dilution level.

For determination of protein powder adulteration, classification models were firstly, developed to classify whey, beef and pea protein powders regardless of their adulterant concentrations or combinations. The second model was developed to classify the adulterant combinations regardless of their protein base or concentrations. For more practical applications where, is it important to merely determine if protein powders are adulterated or not, models were developed to classify all the adulterant mixtures at the lowest adulterant concentration (0.5% w/w) regardless of their protein base. Also, for practical situations where, it may be merely important to determine the type of adulterant in the protein powder and not necessarily its combination with other adulterants, classification models were developed to classify urea, glycine, taurine and melamine separately in adulterated whey protein powder samples, beef protein powder samples and pea protein powder samples. All the classification models described were performed for each of the three setups: benchtop spectrophotometer with optical glass, handheld spectrophotometer with optical glass and handheld spectrophotometer with LDPE plastic bag.

LDA model validations:

The reliability of all the described LDA models was tested by splitting the data into two groups: the training set and validation set. The training set consisted of two-third of the data which included spectra (or sensor signals for datasets from the e-tongue) from the first and second replicate samples. The validation set consisted of spectra (or sensor signals for datasets from the e-tongue) from the third replicate samples. Cross-validation was done three times for each instrumental setup (three-fold cross-validation). The statistical parameters used to evaluate the performance of the LDA models were the recognition accuracy (%) and prediction accuracy (%). Recognition accuracy (%), represents the accuracy of calibration, whereas prediction accuracy (%), represents the accuracy of cross-validation (%). These were assessed through confusion matrices where, columns represented the actual class membership and the rows represented the

predicted class membership. Where visual discrimination was prominent, classification plots of the model was also presented and discussed.

4.3.4. PLSR models for determination of wine, meat and protein powder adulteration

For determination of Tokaji wine adulteration, prediction models were developed to quantitate sugar concentrations of the authentic wines using e-tongue, benchtop spectrophotometer and handheld spectrophotometer.

For determination of optimal dilution for meat analysis with e-tongue, prediction models were also developed to predict the concentration of turkey in the poultry meat mixtures for each of the three tested dilution levels: 0.5%, 1%, and 2% w/v aqua dilution.

For determination of optimal extraction method for meat analysis with e-tongue, prediction models were also developed to predict turkey and beef concentration in poultry and red meat mixtures respectively for all the three tested extraction methods: raw meat, frozen meat and cooked meat extraction method.

For determination of protein powder adulteration, prediction models were developed to predict the concentrations of urea, glycine, taurine and melamine in all the protein powder mixtures regardless of their protein base, mixture combination and adulterant concentration, using all three setups: benchtop spectrophotometer, handheld spectrophotometer with optical glass and handheld spectrophotometer with LDPE plastic bag. For more robust models, independent predictions were also performed to predict urea, glycine, taurine and melamine in all the protein powder mixtures regardless of their protein base, mixture combination and adulterant concentration. For this, the dataset from each of the three setups was repartitioned by splitting into two groups: the training set and validation set. The training set consisted of the first and second replicates of each mixture sample whereas, the validation set consisted of third replicate of each mixture sample (this was never used in the calibration model). Independent prediction was only performed with the dataset from the protein powders due to its data matrix. For practical situations where it may be important to merely predict protein powder concentrations, two additional independent prediction model was developed for each setup firstly, to predict protein powder concentrations regardless of the protein base, adulterant combination or adulterant concentration and secondly to predict protein powder concentrations in protein powder samples containing only single adulterants and regardless of their protein base or adulterant concentrations.

PLSR model validations:

The predictive significance of all the PLS regression models described was tested with leave-one-sample out cross-validations: spectra (for NIRS analysis) or sensor signal (for e-tongue analysis) of all three repeats of a sample (9 spectra/sensor signals) was each time, left out of the

validation process. The statistical parameters used to evaluate the performance of the PLS regression models were the root mean square error of calibration (RMSEC) and the coefficient of determination (R^2C); in cross-validation (RMSECV, R^2CV). The accuracies for the independent predictions were reported as determination coefficient of prediction (R^2pred) and root mean square error of prediction (RMSEP). For all the models, the optimum number of latent variables was determined based on the minimum, RMSEC, RMSECV and RMSEP value to prevent over fitting of the models. The "aquap2" package (Kovacs and Pollner 2016) in R-project was used for all spectral evaluations.

4.3.5. Limit of detection, limit of quantification and limit of quantification for determination of protein powder adulteration

For the protein powder mixtures, limit of detection minimum value (LODmin), limit of detection maximum value (LODmax), limit of quantification minimum value (LOQmin), limit of quantification maximum value (LOQmax), explained variance X (the actual dataset) and explained variance Y (the predicted dataset) were calculated through the partial least-squares (PLS) methods according to the International Union of Pure and Applied Chemistry (IUPAC) approach described by (Allegrini and Olivieri 2014):

$$LODmin = 3.3\,[SEN - var\,(x) + h0min\,SEN - var\,(x) + h0min\,var\,(ycal)]1/2$$

$$LODmax = 3.3\,[SEN - var\,(x) + h0max\,SEN - var\,(x) + h0max\,var\,(ycal)]1/2$$

Where, SEN is the sensitivity (inverse of the length of the regression coefficient), var (x) is the variance of the instrument signals. h0min/max is the minimum/maximum distance between a hyperplane for the calibration set, representing the scores of the samples for which the analyte of interest is absent and the center of a normalized calibration score space. Var (ycal) is the variance in the calibration concentrations. Lower and upper limits of the LODmin/max interval (LODmin and LODmax) correspond to the calibration samples with the lowest and largest extrapolated leverages to zero analyte concentration (Lukacs *et al.*, 2018). LOQmin/max interval was obtained by multiplying the LODmin/max values with a factor value of three (Allegrini and Olivieri 2014). LODmin/max and LOQmin/max values were used to further evaluate the performance of the models for detecting urea, glycine, taurine and melamine in protein powders using all three setups: benchtop spectrophotometer with optical glass, handheld spectrophotometer with optical glass and handheld spectrophotometer with LDPE plastic bag.

5. RESULTS AND DISCUSSION

The results from all the experiments in the materials and methods section are presented and discussed in this section. For clarity and easy understanding, the results from each instrument are discussed according with the type of food adulteration that was investigated. Results from the application of NIRS and e-tongue for the determination of Tokaji wine are discussed first, followed by the determination of optimal dilution and optimal extraction method for meat analysis with e-tongue, then finally discussions of NIRS for the detection of whey, beef and pea protein powder adulteration.

5.1. Determination of Tokaji wine adulteration with NIRS

Discussed in this section are the results from NIRS spectra examination, preprocessing, LDA and PLSR results for determination of Tokaji wine adulteration.

5.1.1. NIRS spectra examination and optimization

Based on the location of the absorption peaks of the tested components and results of PCA, the wavelength ranges of 950–1650 nm was selected for the spectra optimization using benchtop (Figure 17 B). The spectra of handheld spectrophotometer were observed to be characterized by clipping between the wavelength range 1400-1500 nm (Figure 17 C), so the wavelength range 950-1400 nm was selected (Figure 17 D) to eliminate the clipping before spectra optimization.

There was no drastic change in temperature throughout the analysis as temperature ranged from 24.5-26.5 °C throughout the experiment. However, other environmental characteristics, physical characteristics, detector sensitivities and spectra collection methods are some of the many factors that can affect reflectance or diffuse reflectance measurements especially at higher wavelengths (combination bands region) (Agelet and Hurburgh 2010). From Figure 17 (A and C), the spectra from both benchtop and handheld spectrophotometer showed baseline offsets. The baseline offset was suspected to be due to the large distance from the unit's light sources to the reflective/diffusive backing of the transflectance cell. Handheld spectrophotometer had the highest baseline offset. The observed baseline offsets suggested that they may require some form of spectra correction.

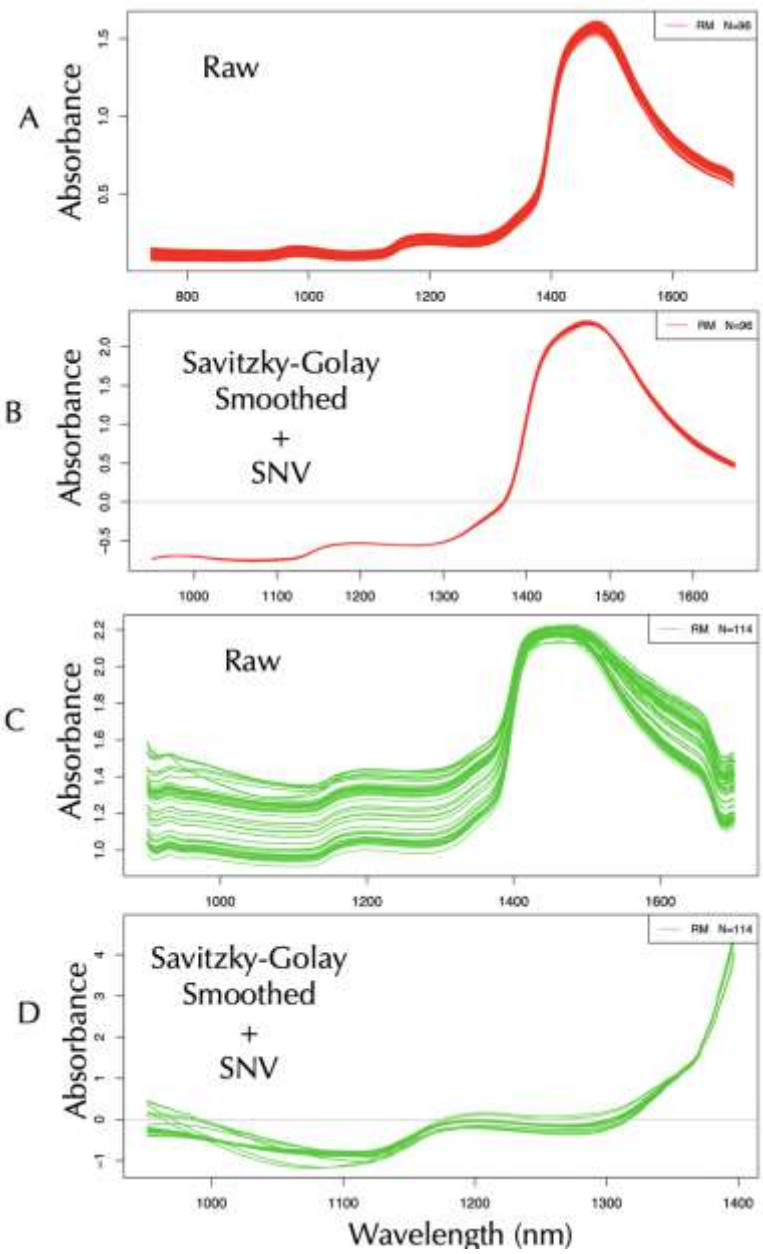

Figure 17: Raw spectra (A) and pretreated spectra (B) plot from the benchtop spectrophotometer and raw spectra (C) and pretreated and truncated spectra (D) of the handheld spectrophotometer for the determination of Tokaji wine adulteration. SNV: standard normal variate

Table 6 shows the results of LDA models built for the classification of authentic Tokaji wines using the benchtop spectrophotometer for the different tested pretreatments. Savitzky-Golay smoothing (21 points) followed by standard normal variate (SNV) showed the highest classification accuracy for both the benchtop and handheld spectrophotometers so this was applied before detecting outliers and developing detailed LDA and PLSR models for the determination of Tokaji wine adulteration.

Table 6: Pretreatment selection for Tokaji wine mixtures obtained using the benchtop and handheld spectrophotometer. Wavelength range of 950-1650nm for benchtop spectrophotometer and 950-1400 nm for handheld spectrophotometer

	Benchtop Spectrophotometer		Handheld Spectrophotometer	
	Average recognition (%)	Average prediction (%)	Average recognition (%)	Average prediction (%)
Raw	98.71	91.95	69.85	62.23
Savitzky-Golay smoothing (21 points)	99.58	91.39	71.20	63.98
MSC	91.02	86.36	63.21	54.98
Savitzky-Golay smoothing (21 points) + MSC	98.73	90.65	69.00	60.66
SNV	97.87	86.37	56.93	54.19
Savitzky-Golay smoothing (21 points) + SNV	**100**	**91.95**	**76.68**	**64.41**
1st Derivative	100	89.26	73.60	68.50
1st Derivative + MSC	100	90.21	60.28	54.38
2nd Derivative	99.54	87.01	75.21	58.39
2nd Derivative + MSC	99.15	86.31	60.27	54.38
Detrend	100	84.67	66.57	59.53

5.1.2. Classification of authentic Tokaji wines with benchtop and handheld spectrophotometers

Figure 18 shows the classification of authentic Tokaji wines using the benchtop and handheld spectrophotometer. The lower grade wines (Tokaji Forditas I and Forditas II) could be visually separated from the higher-grade wines (Tokaji Aszu I and Aszu II) using the benchtop spectrophotometer (Figure 18 A). Tokaji Aszu I and Aszu II, which were prepared from first and second class botrytized berries respectively, were classified closely to each other. Tokaji Forditas I and Forditas II, which were prepared by reusing the first- and second-class berries were also classified close to each other.

Using the handheld spectrophotometer (Figure 18 B) showed some visual overlapping. Using the handheld spectrophotometer, all the authentic wines could be classified with 100% correct accuracy after cross-validation except the wine with the highest grade (Aszu I) (Table 7). Aszu I wine was misclassified as Aszu II and Forditas I wines with misclassified 11.04% misclassification rates each.

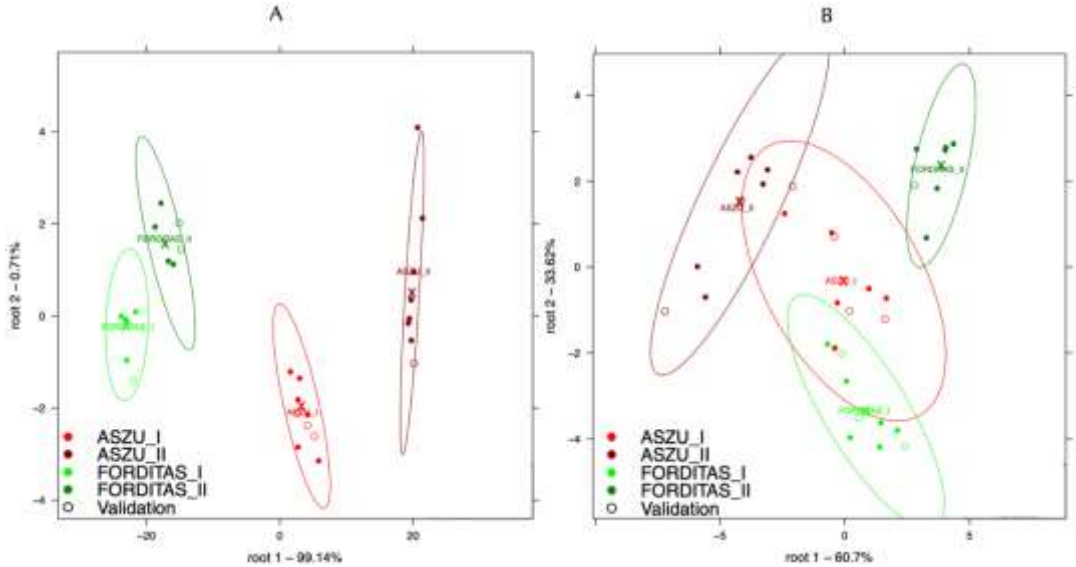

Figure 18: Classification plots developed with the benchtop (A) and the handheld (B) spectrophotometers for classifying authentic Tokaji Aszu I, Aszu II, Forditas I, Forditas II wines

Table 7: Confusion matrix for the classification of authentic Tokaji wines using the handheld spectrophotometer. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%).

Average accuracies		Aszu I	Aszu II	Forditas I	Forditas II
Recognition 98.62%	Aszu I	94.5	0	0	0
	Aszu II	0	100	0	0
	Forditas I	5.5	0	100	0
	Forditas II	0	0	0	100
Cross-validation 94.48%	Aszu I	77.93	0	0	0
	Aszu II	11.04	100	0	0
	Ford I	11.04	0	100	0
	Ford II	0	0	0	100

5.1.3. Classification of authentic Tokaji wines and Forditas I adulterated wines with benchtop and handheld spectrophotometers

Using the benchtop spectrophotometer, there was average recognition of 97.01% and average prediction of 96.78% for the classification of authentic and Forditas I wine that was adulterated with grape must concentrate and sucrose to mimic the sugar concentration of the authentic wines. This could be visualized in Figure 19 with 96.6% of the between group variance

expressed in the root1 of the LDA plot. With cross validation, all the authentic wines could be classified with 100% accuracy, only adulterated Forditas I wines showed misclassification amongst themselves (Appendices - A2, Table 30).

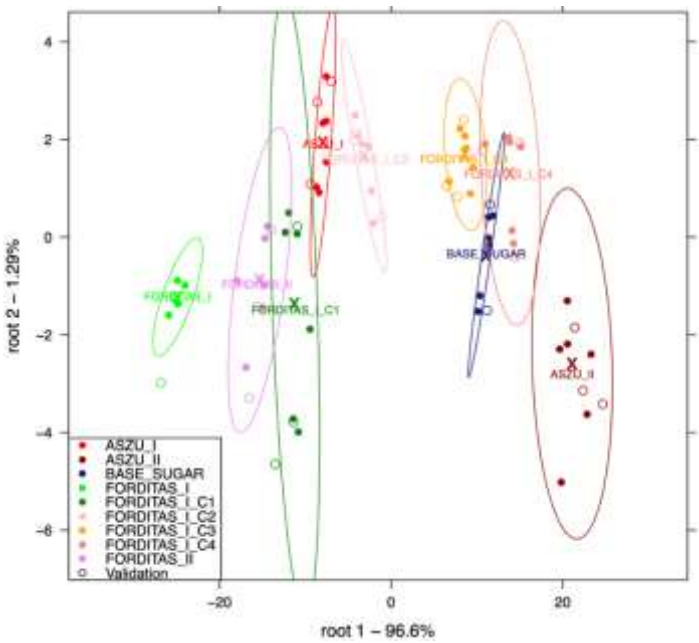

Figure 19: Classification plot developed with the benchtop spectrophotometer for classification of authentic Tokaji wines and Forditas I wine that was adulterated with grape must concentrate and sucrose to mimic the sugar concentration of the authentic wines

Using the handheld spectrophotometer, there was average recognition of 72.40% and average prediction of 68.22% for the classification of authentic and Forditas I wine that was adulterated with grape must concentrate and sucrose to mimic the sugar concentration of the authentic wines. With cross validation, Aszu I, Aszu II, Forditas I and Forditas II could be classified 70.18%, 77.93%, 89% and 66.67% accuracy respectively. The wine that contained sucrose before refermentation (Base sugar wine) was misclassified as authentic Aszu I wine and authentic Aszu II wine with misclassification rates of 33.44% and 11.04% respectively. Adulterated Forditas I wines concentrate also showed misclassification amongst themselves (Appendices – A2, Table 31). There was no clear visual pattern in the classification plot of the handheld spectrophotometer.

5.1.4. Classification of authentic Tokaji wines and Forditas II adulterated wines with benchtop and handheld spectrophotometers

Using the benchtop spectrophotometer, there was average recognition of 98.76% and average prediction of 98.78% for the classification of authentic and Forditas II wine that was adulterated with grape must concentrate and sucrose to mimic the sugar concentration of the authentic wines. This could be visualized in Figure 20 with 96.47% of the variance expressed in

the root1 of the LDA plot. With cross validation, all the authentic wines could be classified with 100% accuracy, only adulterated Forditas II wines showed misclassification amongst themselves (Appendices -A3, Table 32).

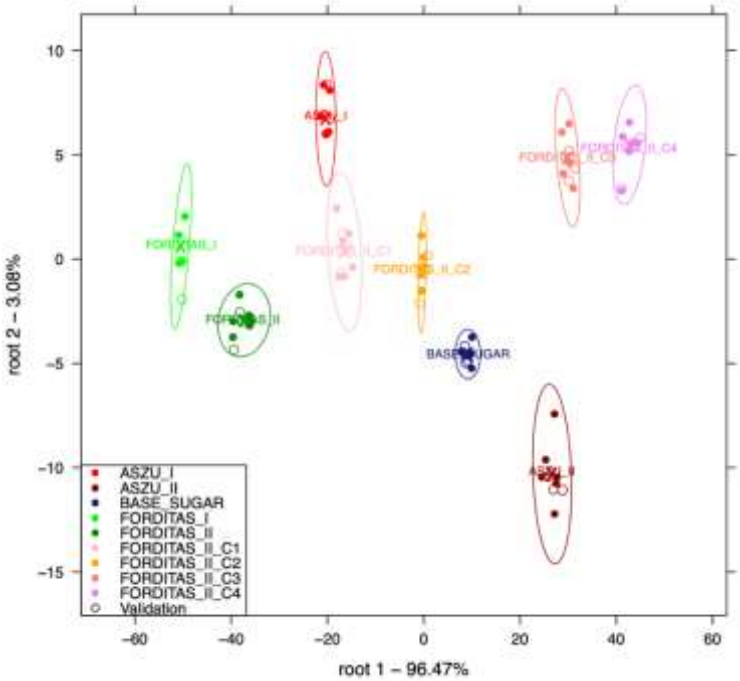

Figure 20: Classification plot developed with the benchtop spectrophotometer for classifying authentic Tokaji wines and Forditas II wine that was adulterated with grape must concentrate and sucrose to mimic the sugar concentration of the authentic wines

Using the handheld spectrophotometer, there was average recognition of 81.87% and average prediction of 76.07% for the classification of authentic and Forditas II wine that was adulterated with grape must concentrate and sucrose to mimic the sugar concentration of the authentic wines. With cross validation, Aszu I, Aszu II, Forditas I and Forditas II could be classified 80.18%, 89%, 100% and 44.48% accuracy respectively. The wine that contained sucrose before refermentation (Base sugar wine) was misclassified as authentic Aszu I wine and authentic Aszu II wine with misclassification rates of 33.44% and 11.04% respectively. The lowest grade authentic wine (Tokaji Forditas II wine) was misclassified as authentic Forditas I wine and base sugar wine with misclassification rates of 33.44% and 11.04% respectively. Forditas II wines showed misclassification amongst themselves (Appendices -A3, Table 33). There was no clear visual pattern in the classification plot of the handheld spectrophotometer.

5.1.4.1. PLSR prediction of Tokaji wine sugar concentrations with NIRS

Sugar concentrations of authentic wines were predicted in PLSR with both benchtop and handheld spectrophotometer (Table 8).

Table 8: PLSR models developed with the benchtop and handheld spectrophotometer to predict sugar concentrations of Tokaji wines

Instrument	Wavelength range (nm)	Latent Variable (LV)	R^2C	RMSEC (g/L)	R^2CV	RMSECV (g/L)
Benchtop spectrophotometer	950-1650	10	0.99	2.72	0.92	16.80
Benchtop spectrophotometer	950-1400	10	0.98	6.73	0.45	46.59
Handheld spectrophotometer	950-1400	10	0.91	17.21	NR	194.73

NR: not reliable

Benchtop spectrophotometer could predict the sugar concentration of the authentic wines with R^2CV of 0.92 and RMSECV of 16.80 g/L using the wavelength range between 950 and 1650 nm. The handheld spectrophotometer could not predict the sugar content of the authentic wines with cross-validation although calibration models were possible. When PLSR models were developed for the benchtop spectrophotometer using a wavelength range of 950-1400 nm, lower accuracies were achieved with R^2CV 0.45 and RMSECV 46.59 g/L. This suggests that the first overtone wavelength range of water (1300-1600 nm) maybe, provides important information for tracking Tokaji wine adulteration with grape must concentrate as already reported in studies related to other liquids (Muncan 2019) through the novel scientific discipline known as “aquaphotomics” (Tsenkova et al. 2018). The interval between 1441 and 1470 nm have been assigned to the water dimers, trimers and tetramers i.e. water molecules having H-bonds with one, two or three other water molecules, respectively (Luck 1998). Bands above 1500 nm have been related to aqueous solutions of fructose (1583 nm), sucrose (1584 nm) and glucose (1587 nm) (de Almeida et al. 2018). Some of these sugars, have been reported to be major constituents in grape must concentrate composition (Coelho et al. 2007).

The results from this study signal a potential for using near infrared spectroscopy to track Tokaji wine adulteration. For practicality on industrial basis, further studies may be required with higher sample numbers for more robust models. The setup of the handheld spectrophotometer used in this study should also be carefully considered and adapted to better experimental procedures if it is to be used to track Tokaji wine adulteration.

5.2. Determination of Tokaji wine adulteration with e-tongue

Discussed in this section, are the results from e-tongue sensor optimization, LDA and PLSR analysis for determination of Tokaji adulteration.

5.2.1. E-tongue signal correction

All the seven e-tongue sensors in the Alpha Astree liquid and taste analyzer often exhibit a combined effective for the determination of food quality (Soós et al. 2015; Chung et al. 2019; F. A. Koncz et al. 2017). However, the sensors respond differently to environmental conditions that could arise during analysis and may influence sensor sensitivity (Panchuk et al. 2016).

5.2.2. Classification of authentic Tokaji wines with e-tongue

All the authentic wines could be visually separated from each other (Figure 21). There was 100% cross-validation accuracy for all the different authentic wines using the e-tongue.

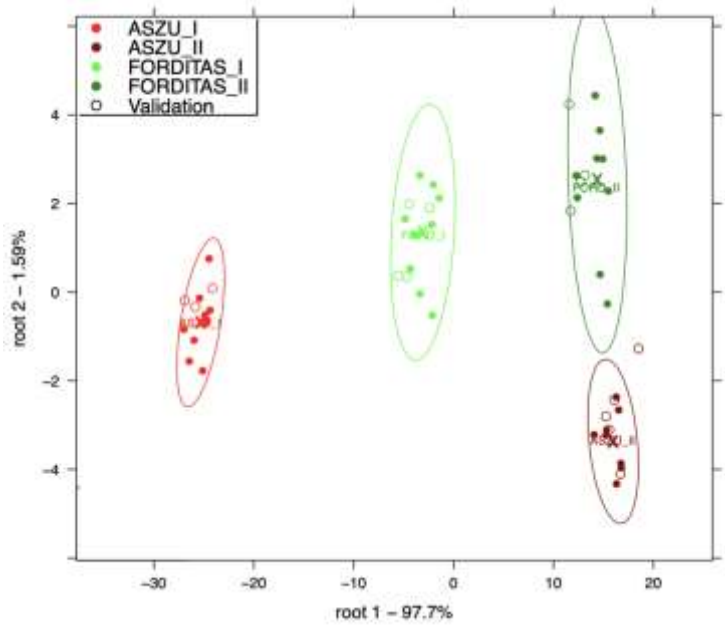

Figure 21: Classification plot developed with the e-tongue for classifying authentic Tokaji Aszu I, Aszu II, Forditas I, Forditas II

Figure 22 (A) shows the e-tongue classification plots of authentic and Forditas I wine that was adulterated with grape must concentrate and sucrose to mimic the sugar concentration of the authentic wines. Visually, all the wine mixtures could be separated and classification was observed in a linear pattern for adulterated wines from C1 to C4 concentration of adulteration. There was average recognition accuracy of 99.54% and prediction accuracy of 98.17% when authentic wines and Forditas I adulterated wines were classified. With cross validation, all the authentic wines could be classified with 100% accuracy, only Forditas I adulterated wines showed misclassification amongst themselves (Appendices – A4, Table 34).

Figure 22 (B) shows the classification plot of authentic and Forditas II wine that was adulterated with grape must concentrate and sucrose to mimic the sugar concentration of the authentic wines. Visually, most of the wine types could be separated but base sugar adulterated wine overlapped with authentic Forditas II wine. There was average recognition of 100% and prediction of 93.17%. The overlapping observed in the classification plot was confirmed by 53.81% misclassification of authentic Forditas II wine as base sugar wine and 7.62% misclassification of base sugar wine as authentic Forditas II wine rate of 53.81% with cross-validation (Appendices – A4, Table 35). This suggests that adding sucrose to wine before refermentation could be a potent form of wine adulteration base sugar adulteration of Forditas II wines could be a potent form of adulteration.

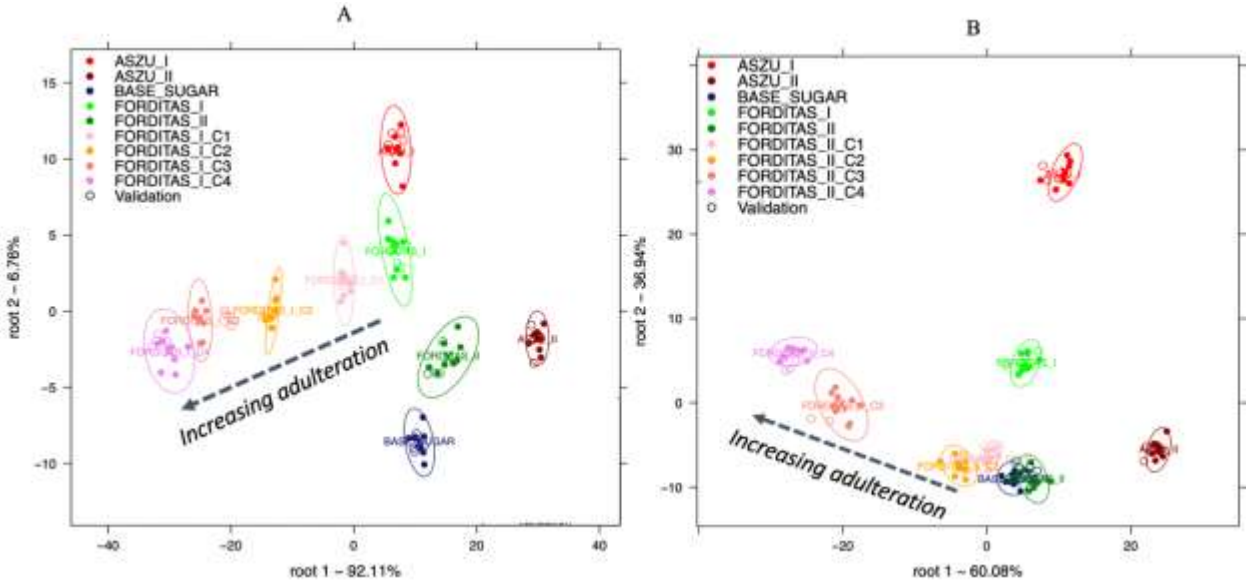

Figure 22: Classification plot developed with the e-tongue for classifying authentic Tokaji wines and Forditas I (A) and Forditas II (B) wines that were adulterated with grape must concentrate and sucrose to mimic the sugar concentration of the authentic wines

Hitherto this study, there was no report about using the e-tongue to detect adulteration of wines with grape must concentrate. The demonstrated classification capabilities of the e-tongue could be taken advantage of by the wine industries for reliable quality monitoring of botrytized wines. Additive correction relative to all samples improved the classification results of the e-tongue datasets (Appendices - A5, Table 36) and can be adopted for samples measurements performed with the same sample set and sequence.

5.2.3. PLSR prediction of Tokaji wine sugar concentrations with e-tongue

Sugar concentration of the wines could be predicted with R^2CV of 0.90 and RMSECV of 17.67 g/L of wine using 7 latent variables. Some studies have also reported similar prediction accuracies when e-tongue was used to predict different wine parameters such as aging

(Rudnitskaya et al. 2007), fermentation compounds (Kutyła-Olesiuk et al. 2014), sensory parameters (Cetó et al. 2015), and characterization of different wines (Buratti et al. 2004).

5.2.4. Performance comparison of benchtop spectrophotometer, handheld spectrophotometer and e-tongue for classifying Forditas I and Forditas wine adulteration

The e-tongue gave the best classification accuracies for the determination of Forditas I adulteration (

Figure 23 A) and the benchtop spectrophotometer gave the best accuracies for the determination of Forditas II wine adulteration (

Figure 23 B). Thus comparatively, the e-tongue could classify Forditas I wines better than the benchtop spectrophotometer but the benchtop spectrophotometer could classify Forditas II wines better. This suggests that the benchtop spectrophotometer was more sensitive to higher added sugar concentrations than the e-tongue as Forditas II adulterated wines contained more grape must concentrate because they were the lowest grade wines.

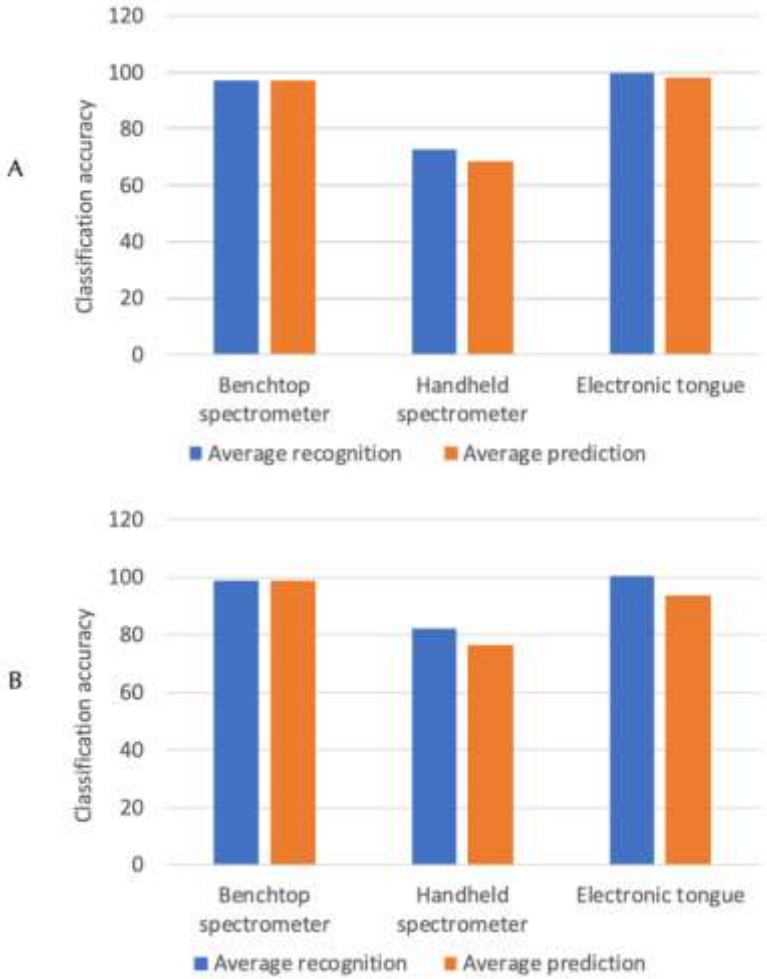

Figure 23: Classification performance comparison of the benchtop spectrophotometer, handheld spectrophotometer and e-tongue for the detection of Tokaji Forditas I wine adulteration (A) and Forditas II wine adulteration (B)

5.3. Determination of optimal dilution for meat analysis

Discussed in this section are the results from e-tongue sensor optimization LDA and PLSR for the determination of optimal dilution and optimal extraction for meat analysis.

5.3.1. E-tongue signal correction

Table 9 shows the sensors that were selected for each of the meat analysis based on the sensor optimization method described in the materials and methods section. Generally, sensors HA, BB, ZZ, GA and JB provided the best accuracies using LDA simulations.

Table 9: Results of LDA sensor optimization using all the three different sample preparation methods to detect turkey/chicken and beef/pork adulteration

Meat combination	Sample preparation	Selected sensors	Omitted sensors	Initial cross-validation accuracies (%)	Optimized cross-validation accuracies (%)
Chicken and turkey adulteration	Raw meat extraction with distilled water	HA, BB, ZZ, GA	JE, CA, JB	47.99	58.35
	Meat extraction by cooking with distilled water	BB, ZZ, GA, JB	HA, JE, CA	54.14	64.72
	Frozen meat extraction with distilled water	All: HA, BB, ZZ, GA, JE, JB, CA	None	62.55	62.55
Pork and beef adulteration	Raw meat extraction with distilled water	HA, ZZ, GA, JB	BB, CA, JE	45.90	54.25
	Meat extraction by cooking with distilled water	HA, ZZ, GA CA, JE, JB	BB	58.37	68.77
	Frozen meat extraction with distilled water	HA, ZZ, BB, GA, JE, JB	CA	52.11	56.41

From PLSR simulations in the sensor optimization process, sensors HA, BB, ZZ and GA were the most important sensors in predicting the concentrations of the meat mixtures. These

sensors provided the lowest RMSECV's (Table 10). This was in agreement with those obtained from sensor optimization for LDA analysis.

Table 10: Results of PLS regression sensor optimization using all the three different extraction methods to detect turkey/chicken and beef/pork adulteration

Meat combination	Sample preparation	Selected sensors	Omitted sensors	Initial RMSECV (% w/w meat mixture)	Optimized RMSECV (% w/w meat mixture)
Turkey and chicken adulteration	Raw meat extraction with distilled water	HA, BB, ZZ, GA,	JE, JB, CA	3.68	3.34
	Meat extraction by cooking with distilled water	HA, BB, ZZ, CA, JB	JE, GA	5.19	4.93
	Frozen meat extraction with distilled water	HA, BB, ZZ, GA, JE	JB, CA	3.04	2.89
Beef and beef pork adulteration	Raw meat extraction with distilled water	HA, BB, CA, GA	JE, JB, ZZ	5.91	5.51
	Meat extraction by cooking with distilled water	HA, ZZ, JB	JE, GA, BB, CA	4.44	3.83
	Frozen meat extraction with distilled water	HA, BB, ZZ JB, JE	GA, CA	5.81	5.16

5.3.2. Classification of poultry meat mixtures after 0.5%, 1% and 2% w/v extract dilution

Figure 24, shows the LDA model developed to classify minced chicken in turkey using all the three different dilution levels. There were visually distinct separation patterns using all three different dilutions. The visual separation was confirmed with average recognition and prediction accuracy of 100% respectively for the classification of adulterated meat samples using all three different dilutions. There was therefore a need to apply some other multivariate tools to ascertain the optimum dilution level. PLS regression was used for this.

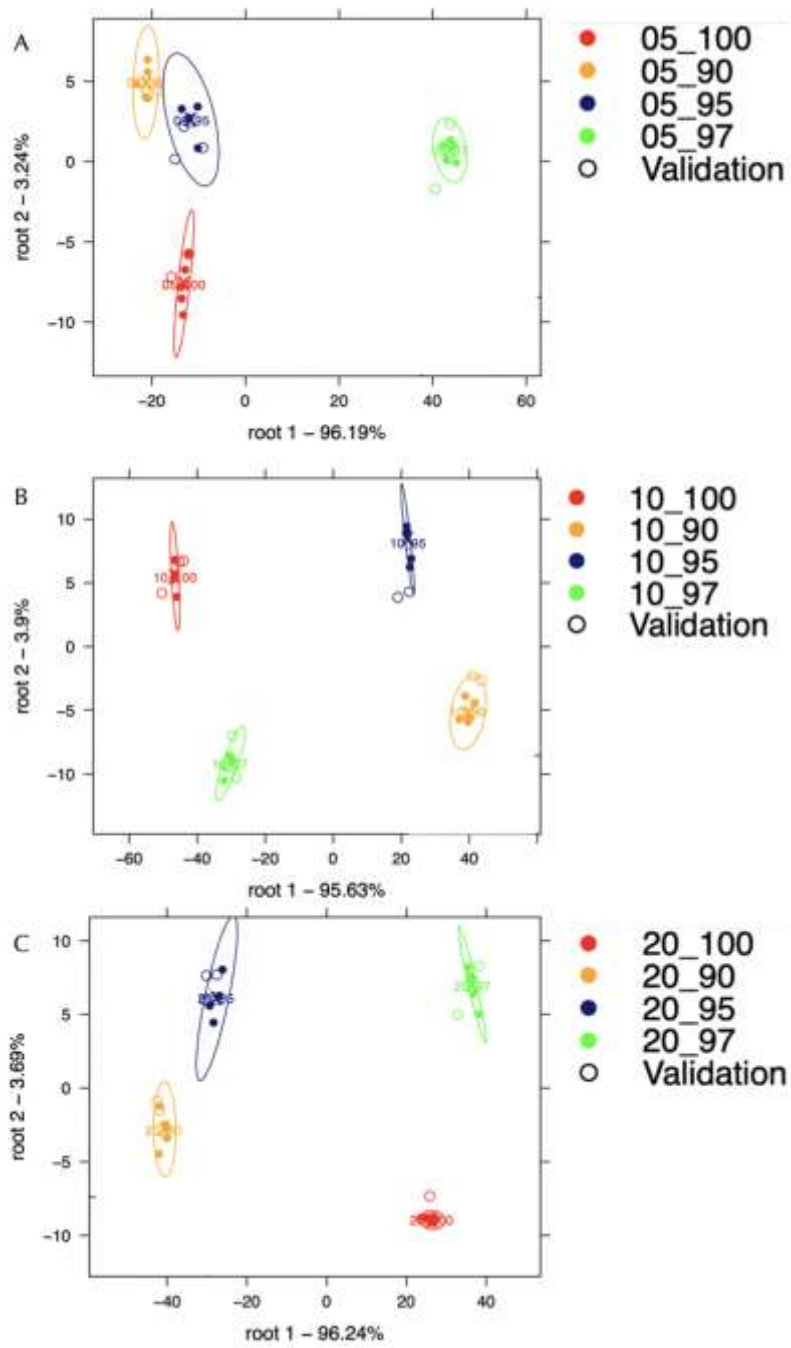

Figure 24: Classification of 100%, 97%, 95% and 90% w/w turkey/chicken using 0.5% (A), 1% dilution (B) and 2% w/v dilution (C)

5.3.3. PLSR predictions of meat mixture concentrations after 0.5%, 1% and 2% w/v extract dilution

All the different dilution levels produced 100% classification accuracy in LDA, so it was necessary to build PLS regression models as well to determine the optimal dilution as shown in Table 11.

Table 11: PLSR models developed with e-tongue to predict concentration turkey in the different meat mixtures after 0.5%, 1% and 2% w/v extract dilution

Dilution level	LV	R^2	RMSEC (% w/w meat mixture)	R^2CV	RMSECV (% w/w meat mixture)
Dilution level 1 (2% w/v turkey)	3	0.88	1.26	0.81	1.57
Dilution level 2 (1% w/v turkey)	3	0.97	0.59	0.95	0.80
Dilution level 3 (0.5% w/v turkey)	1	0.71	1.96	0.65	2.14

The different meat mixtures could be predicted with R^2CV in the range of 0.65-0.95 and RMSECV generally, less than 2.14% w/w of turkey using all three dilutions. Dilution level 2 with 1% w/v was proven to be the optimum dilution level the highest R^2CV of 0.95 and the lowest RMSECV of 0.80% w/w among all the three tested dilution methods. It was used for subsequent experiments for the determination of optimal extraction method.

5.4. Determination of optimal extraction method for meat analysis

5.4.1. Classification of poultry mixtures processed with raw meat extraction

Figure 25 (A), shows the LDA plot developed to classify chicken and turkey adulteration with more than 88% of the between groups variance expressed in the root 1 whereas, Figure 25 (B), shows the LDA plot developed to classify pork and beef adulteration with more than 51% of the variance expressed in the root 1. Very little visual separation could be observed in the plots developed to classify turkey/chicken mixture concentrations but sample T080 could be separated from the other concentrations (Figure 25 A). There was no visual separation in the plot developed to classify beef/pork meat mixtures (Figure 25 B).

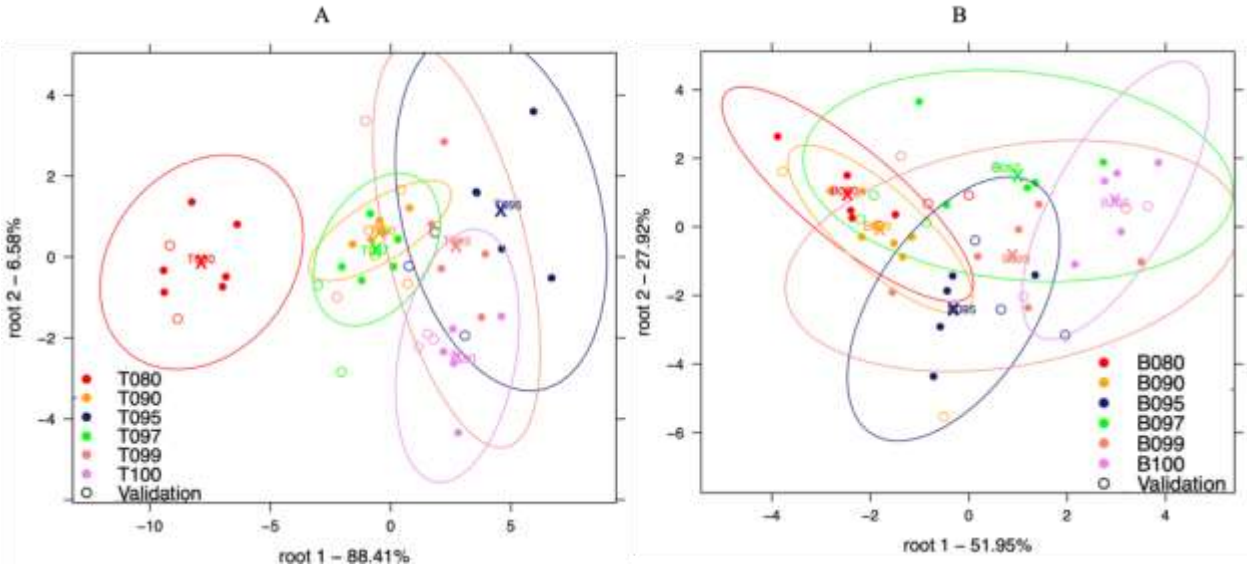

Figure 25: Classification plot developed using e-tongue to classify turkey/chicken mixtures (A) and beef/pork mixtures (B) after the raw meat extraction

Table 12, shows the confusion table for classification of turkey/chicken meat mixture concentrations using the raw meat extraction method. There was average recognition accuracy of 81.28% and prediction accuracy of 58.35%. With cross validation, only sample T080 showed 100% classification, confirming the separation observed in the plot. Samples T090 and T100 showed the second highest classification accuracies of 87.59% each. However, 12.41% of sample T090 was misclassified as T097 and 12.41% of sample T100 was misclassified as T095. Samples T099 and T097 had the lowest correct classification accuracies of 12.36% and 25.09% respectively.

Table 12: Confusion table for the classification of turkey/chicken meat mixture concentrations using the raw meat extraction method. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%). T: percentage of turkey in the mixture

Average accuracies		T080	T090	T095	T097	T099	T100
Recognition 81.28%	T080	100	0	0	0	0	0
	T090	0	93.81	18.73	24.95	0	0
	T095	0	0	56.18	0	12.57	6.19
	T097	0	6.19	0	68.86	6.19	0
	T099	0	0	12.55	6.19	75.05	0
	T100	0	0	12.55	0	6.19	93.81
Cross-validation 58.35%	T080	100	0	0	0	0	0
	T090	0	87.59	0	49.81	0	0
	T095	0	0	37.45	0	25.09	12.41
	T097	0	12.41	12.36	25.09	37.45	0
	T099	0	0	25.09	25.09	12.36	0
	T100	0	0	25.09	0	25.09	87.59

Table 13, shows the confusion table for classification of beef/pork meat mixture concentrations using the raw meat extraction method. There was average recognition accuracy of 67.73% and prediction accuracy of 54.25%. The poor separation in the plot (Figure 25 B) was confirmed by misclassifications in all the mixtures with cross-validation. The worst misclassification was 37.45%, observed for sample B099. B080 gave the best correct classification accuracy of 62.78%. Samples B095 and B097 both gave correct classification accuracies of 50% respectively.

Table 13: Confusion table for the classification of beef/pork meat mixture concentrations using the raw meat extraction method. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%). B: percentage of beef in the mixture

Average accuracies		B080	B090	B095	B097	B099	B100
Recognition 67.73%	B080	75.05	6.19	0	24.95	12.57	0
	B090	6.19	75.05	0	0	0	0
	B095	0	12.57	75.05	0	18.76	0
	B097	18.76	6.19	6.19	56.29	6.19	0
	B099	0	0	18.76	12.57	43.71	18.76
	B100	0	0	0	6.19	18.76	81.24
Cross-validation 54.25%	B080	62.78	12.41	0	25.19	25.09	0
	B090	12.41	62.78	0	12.41	0	0
	B095	0	12.41	50	0	12.36	0
	B097	12.41	12.41	12.41	50	0	0
	B099	12.41	0	37.59	0	37.45	37.45
	B100	0	0	0	12.41	25.09	62.55

Misclassification rates associated with the concentrations 99%, 97% and 95% w/w of both turkey/chicken and beef/pork meat mixtures after raw meat extraction suggests that perhaps, the meat compounds extracted were not sufficient for detection and discrimination with the e-tongue. Extracting meat compounds with water may be a challenge especially with the presence of fat-soluble compounds, which is often processed with other methods such as the Soxhlet method, Bligh and Dyer method, Folch method, microwave solvent extraction etc. (Hewavitharana et al. 2020). This is particularly true as the samples with the highest concentrations: T080 and B080 always gave the best classification accuracies whereas, those with the lowest concentration T099 and B099 consistently gave the worst. Extraction of meat compounds often involve using denaturing or non-denaturing solutions, which can be expensive (Malva et al. 2018). Better accuracies of 100% were however achieved with this extraction for determination of optimal dilution. This could be due to the differences in the range of the mixture concentrations.

5.4.2. Classification of meat mixtures processed with frozen meat extraction method

Very little visual separation could be observed in the classification plots developed to classify turkey/chicken mixture concentrations (Figure 26 A) but sample T080 and T090 could be

distinguished in the plot. In the classification plot developed to classify beef/pork mixture concentrations (Figure 26 B), only B080 and B097 showed some visual separation.

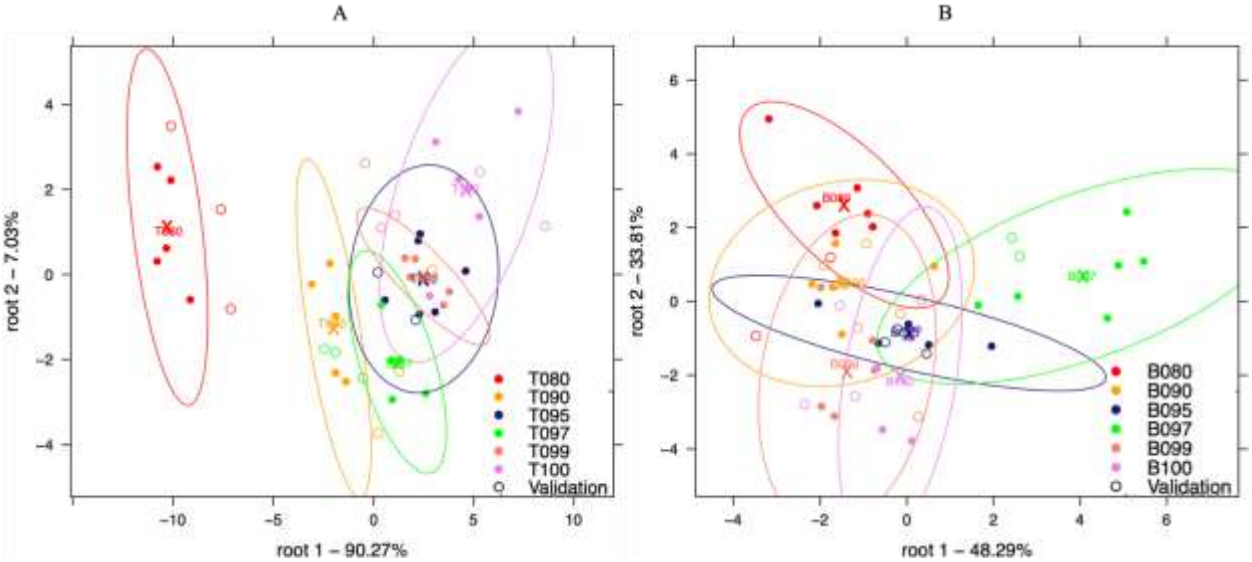

Figure 26: Classification plot developed using e-tongue to classify turkey/chicken mixtures (A) and beef/pork mixtures (B) after frozen meat extraction method

Table 14, shows the confusion table for classification of turkey/chicken meat mixture concentrations using the frozen meat extraction method. There was average recognition of 80.52% and prediction accuracy of 62.55%. With cross validation, only sample T080 showed 100% classification, confirming the separation in the plot. Samples T095, T097 and T100 showed the second highest correct classification accuracies of 62.55%, 62.55% and 75.19%. The worst classification was observed for T099.

Table 14: Confusion table for the classification of turkey/chicken meat mixture concentrations using the frozen meat extraction method. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%). T: percentage of turkey in the mixture

Average accuracies		T080	T090	T095	T097	T099	T100
Recognition 80.52%	T080	100	0	0	0	0	0
	T090	0	81.39	6.19	6.19	0	0
	T095	0	6.2	87.62	0	18.76	0
	T097	0	6.2	0	93.81	0	0
	T099	0	6.2	6.19	0	81.24	12.55
	T100	0	0	0	0	0	87.45
Cross-validation 62.55%	T080	100	0	0	0	0	0
	T090	0	37.45	0	25.09	0	0
	T095	0	25.09	62.55	12.36	50	12.41
	T097	0	25.09	0	62.55	12.41	0
	T099	0	12.36	25.09	0	37.59	12.41
	T100	0	0	12.36	0	0	75.19

Table 15, shows the confusion table for classification of beef/pork using the frozen meat extraction method. There was average recognition accuracy of 85.51% and prediction accuracy of 56.41%. The poor separation in the plot (Figure 26 B) was confirmed by misclassifications in all the mixtures with cross-validation. The worst misclassification was 12.41%, observed for sample B099. Sample B097 gave the best cross-validation accuracy of 87.59%. B080 showed misclassifications with the higher ranged concentrations (B090 and B095) but not with the lower ranged ones (B097 and B099).

Table 15: Confusion table for classification of beef/pork using the frozen meat extraction method. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%). B: percentage of beef in the mixture

Average accuracies		B080	B090	B095	B097	B099	B100
Recognition 85.51%	B080	100	0	0	0	0	0
	B090	0	93.81	12.55	0	6.19	0
	B095	0	0	68.73	0	6.19	12.55
	B097	0	0	6.18	100	0	0
	B099	0	6.19	12.55	0	75.05	12.55
	B100	0	0	0	0	12.57	74.91
Cross-validation 56.41%	B080	50	0	0	0	0	0
	B090	37.59	100	12.41	0	12.41	0
	B095	12.41	0	62.78	12.41	25.19	37.45
	B097	0	0	0	87.59	0	0
	B099	0	0	12.41	0	12.41	37.45
	B100	0	0	12.41	0	50	25.09

Misclassifications observed with the frozen meat extraction method suggest that, the meat compounds extracted may also, not have been sufficient for detection and discrimination by the e-tongue sensors. The quality of frozen/thawed meat is affected by the amount of frozen and unfrozen water, freezing rate and the temperature and time of frozen storage (Daszkiewicz, Kubiak, and Panfil 2018). The characteristics of unfrozen water influences the rate and extent of physical, chemical, and biochemical processes in meat (Leygonie, Britz, and Hoffman 2012) which, may have influenced drip and the concentrations of compounds necessary for the detection and discrimination with the e-tongue sensors.

5.4.3. Classification of meat mixtures processed with cooked meat extraction method

Very little visual separation could be observed in the plot developed to classify turkey/chicken mixtures using the cooked meat extraction method (Figure 27 A). In the plot developed to classify beef/pork mixtures (Figure 27 B), there was a decreasing pattern of mixture concentrations from left to right.

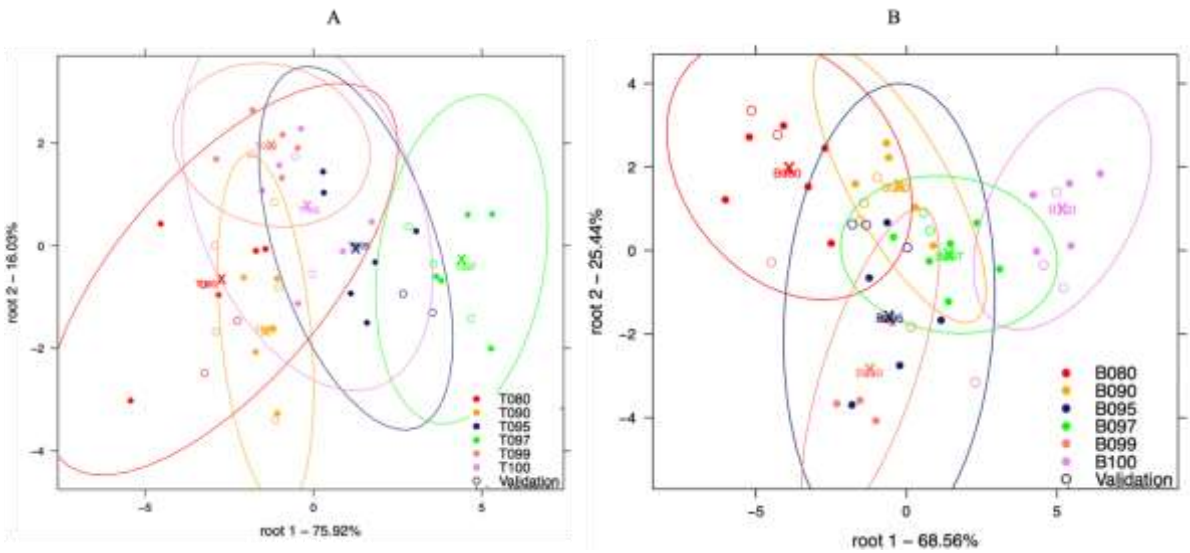

Figure 27: Classification plot developed using e-tongue to classify turkey/chicken mixtures (A) and beef/pork mixtures (B) after cooked meat extraction method

Table 16, shows the confusion table for classification of turkey/chicken meat mixtures using the cooked meat extraction method. There was average recognition accuracy of 78.13% and prediction accuracy of 64.73%. With cross validation, sample T097 yielded the highest classification of 87.59%. Samples T090 and T080 also showed cross-validation accuracies of 75.19% respectively. The worst classifications were observed for T099 with 25.09% cross-validation accuracy.

Table 16: Confusion table for classification of turkey/chicken meat mixtures using the cooked meat extraction method. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%). T: percentage of turkey in the mixture

Average accuracies		T080	T090	T095	T097	T099	T100
Recognition 78.13%	T080	74.91	0	0	0	6.19	0
	T090	12.55	87.62	0	0	6.19	12.55
	T095	0	0	75.05	0	0	12.55
	T097	0	0	0	100	0	0
	T099	12.55	6.19	0	0	68.86	12.55
	T100	0	6.19	24.95	0	18.76	62.36
Cross-validation 64.72%	T080	75.19	0	0	0	12.36	0
	T090	12.41	75.19	0	0	25.09	12.41
	T095	0	0	62.55	12.41	0	12.41
	T097	0	0	12.36	87.59	0	0
	T099	12.41	12.41	0	0	25.09	12.41
	T100	0	12.41	25.09	0	37.45	62.78

Table 17, shows the confusion table for classification of beef/pork meat mixtures using the cooked meat extraction method. There was average recognition accuracy of 89.62% and prediction

accuracy of 68.77%. With cross validation, only sample B100 showed 100% cross-validation accuracy. Samples B090 and B080 showed the second highest correct classification accuracies of 87.59% each and misclassifications of 12.41% each. B080 only showed misclassification with B090 whereas, B090 only showed misclassification with B095. The worst classifications were observed for B095 with 37.45% cross-validation accuracy. B097 and B099 showed correct classification accuracies of 50% each.

Table 17: Confusion table for classification of beef/pork meat mixtures using the cooked meat extraction method. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%). B: percentage of beef in the mixture

Average accuracies		B080	B090	B095	B097	B099	B100
Recognition 89.62%	B080	100	0	0	0	0	0
	B090	0	87.62	18.73	6.19	0	0
	B095	0	6.19	68.73	6.19	6.19	0
	B097	0	6.19	0	87.62	0	0
	B099	0	0	12.55	0	93.81	0
	B100	0	0	0	0	0	100
Cross-validation 68.77%	B080	87.59	0	0	0	12.41	0
	B090	12.41	87.59	25.09	12.41	0	0
	B095	0	12.41	37.45	25.19	25.19	0
	B097	0	0	0	50	12.41	0
	B099	0	0	37.45	12.41	50	0
	B100	0	0	0	0	0	100

Besides time consumption, elevated temperatures have been widely acknowledge to be effective in the extraction of bioactive compounds in diverse foods (Putnik et al. 2018; Khan, Aslam, and Makroo 2019). Meat extraction by cooking was also reported to be effective in the extraction of compounds from chicken bone (Kumoro et al. 2010) and also, for the detection beef adulteration with illegal hormonal substances (Goga, Ferraro, and Barbera 2011).

5.4.4. Performance comparison of three extraction methods for classifying turkey/chicken and beef/pork mixtures with e-tongue

Comparatively, all the three extraction methods yielded similar recognition accuracies but the cooked meat extraction yielded the best cross-validation accuracies for classifying turkey/chicken mixtures (Figure 28 A) and beef/pork mixtures (Figure 28 B). Better accuracies were achieved for the red meat adulteration compared to the poultry adulteration. Additive correction relative to all samples also improved the classification results of all the datasets (Appendices - A6, Table 37)

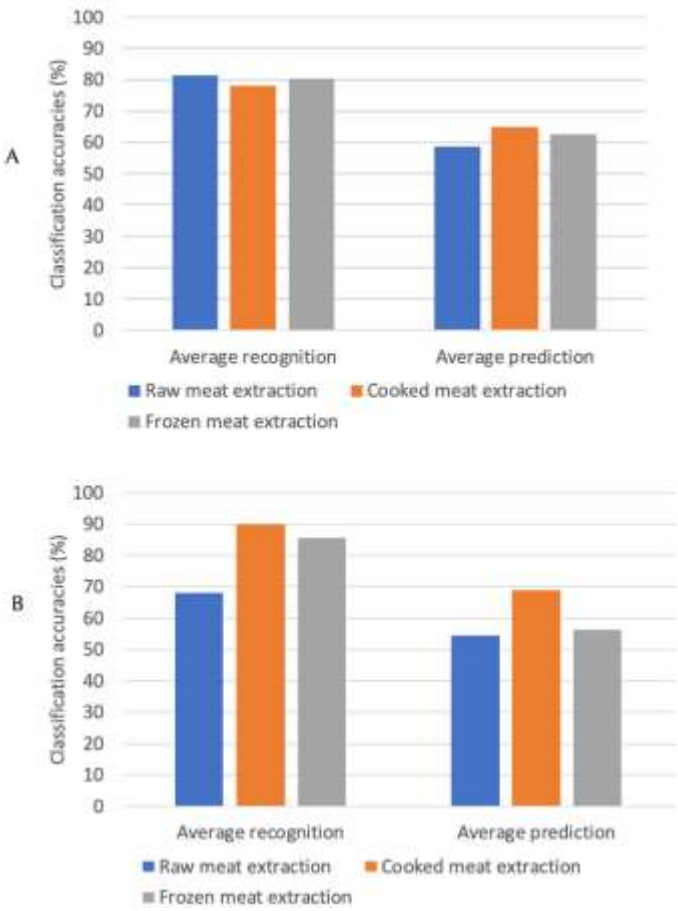

Figure 28: Classification performance comparison of the different extraction methods for the classification of poultry meat mixtures (A) and red meat mixtures (B) using e-tongue

5.4.5. PLSR prediction of meat mixtures processed with the three different extraction methods

Table 18, shows the PLS models to regress on adulterated meat mixtures using the raw meat extraction with distilled water, meat extraction by cooking with distilled water and frozen meat extraction with distilled water methods. Using latent variables (LV) in the range of three to five, the different concentrations of meat samples could be predicted with R^2CV's in the range 0.34-0.76 and errors (RMSECV) in range 3.34-5.51% w/w of meat mixtures. The best PLSR model for the prediction of chicken in turkey was achieved when the frozen meat extraction with distilled water method was used but for pork in beef, it was the meat extraction by cooking.

Table 18: PLS models developed to predict the concentration of adulterated meat mixtures using all the three extraction methods

Meat mixture	Sample preparation method	LV	R^2	RMSEC (% w/w meat mixture)	R^2CV	RMSECV (% w/w meat mixture)
Chicken and turkey	Raw meat extraction with distilled water	3	0.82	2.91	0.76	3.34
	Meat extraction by cooking with distilled water	5	0.67	3.92	0.47	4.93
	Frozen meat extraction with distilled water	4	0.86	2.57	0.81	2.89
Pork and beef	Raw meat extraction with distilled water	4	0.51	4.78	0.34	5.51
	Meat extraction by cooking with distilled water	3	0.76	3.35	0.72	3.83
	Frozen meat extraction with distilled water	4	0.65	4.05	0.43	5.16

Figure 29, shows a visual comparison of the three methods based on their PLSR predictions of pork in beef. The extraction by cooking yielded the highest R^2CV and lowest RMSECV results after cross-validation for predicting pork (Figure 29, A) but yielded the worst accuracies in predicting chicken in turkey (Figure 29, B).

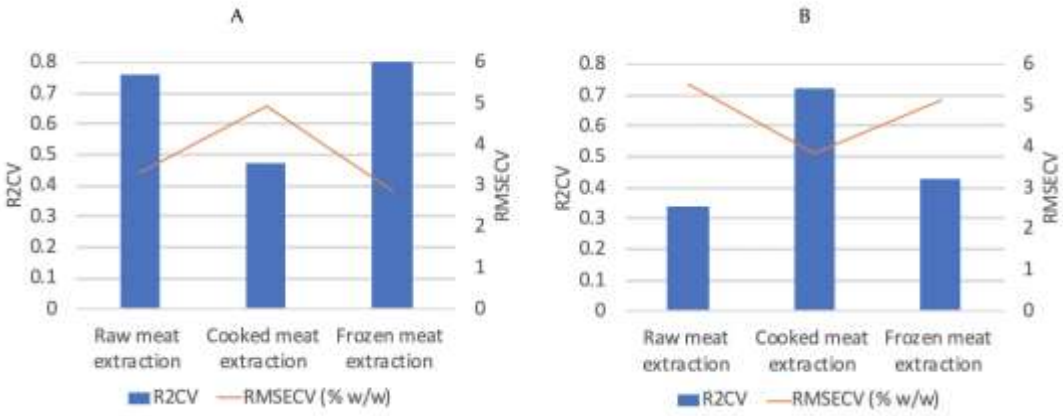

Figure 29: PLS performance comparison of the classification of poultry mixtures (A) and red meat mixtures (B) after electronic tongue analysis using all three extraction methods

5.5. Determination of protein powder adulteration with NIRS

Discussed in this section are the results from NIRS spectra examination, preprocessing, LDA and PLSR for determination of whey, beef and pea protein powder adulteration with urea (U), glycine (G), taurine (T) and melamine (M).

5.5.1. Spectral preprocessing and optimization

Figure 30, shows the raw spectra plot (A) and pretreated spectra plot (B) of whey, beef and pea protein powder using the benchtop spectrophotometer, the raw spectra (C) and pretreated spectra (D) of whey, beef and pea protein powder using the handheld spectrophotometer and optical glass and raw spectra (E) and pretreated spectra (F) of whey, beef and pea protein powder using the handheld spectrophotometer and LDPE plastic bag.

Based on the location of the absorption peaks of the tested components and results of PCA, the wavelength range 950–1650 nm was selected and used for the spectra optimization. This was done for spectra from all three setups (Figure 30 B, D and F): benchtop spectrophotometer and optical glass, handheld spectrophotometer and optical glass and spectrophotometer and LDPE plastic.

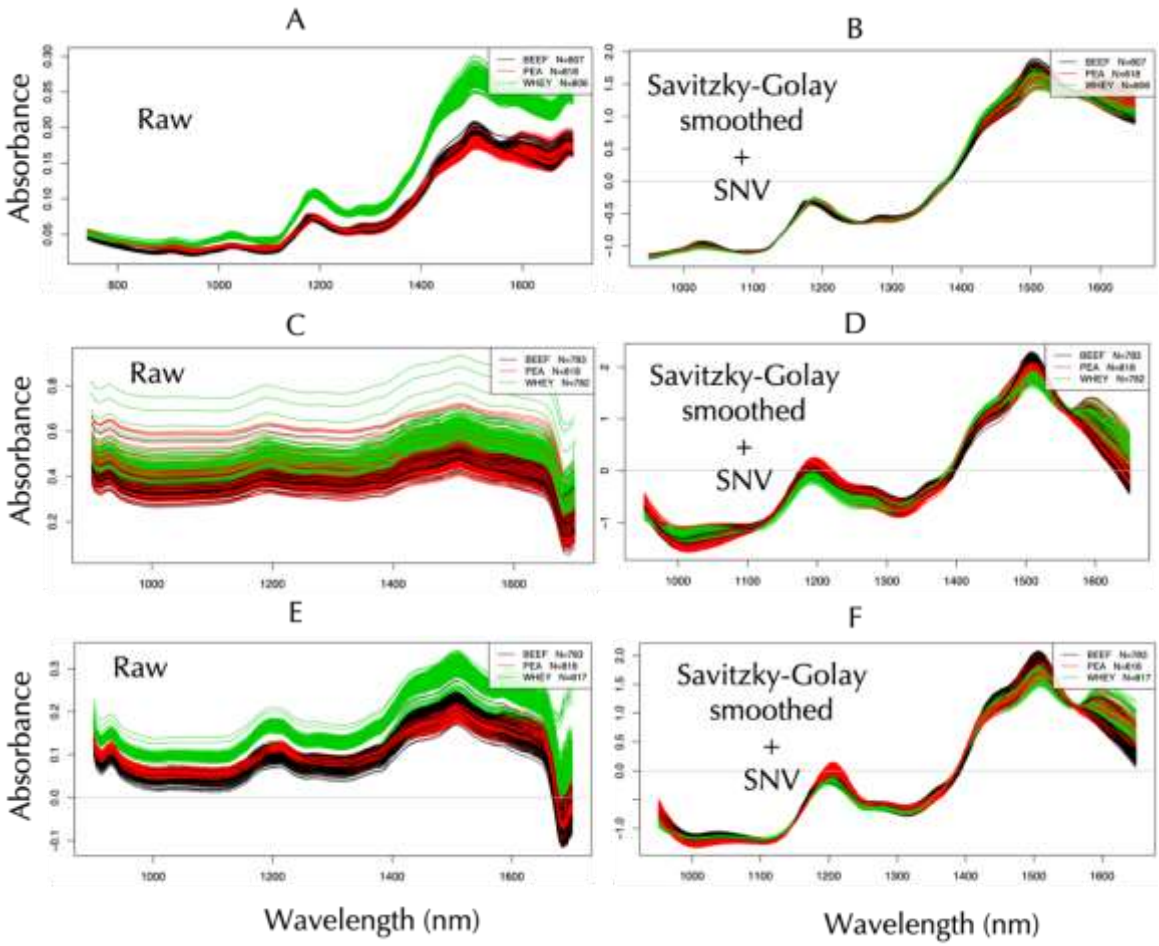

Figure 30: Raw spectra plot of protein powder mixtures using the benchtop spectrophotometer (A), handheld spectrophotometer with optical glass (C) and handheld spectrophotometer with LDPE plastic (E). Pretreated spectra plot of protein powder mixtures using the benchtop spectrophotometer (B), handheld spectrophotometer with optical glass (D) and handheld spectrophotometer with LDPE plastic (F). SNV: standard normal variate

The handheld spectrophotometer scanned through the optical glass had the highest base line shift (Figure 30 C) compared to the one scanned through the LDPE plastic bag (Figure 30 E) or when benchtop spectrophotometer (Figure 30 A) was used. The reason for this could be that, the optical glass surface could not fit properly to the window of the handheld spectrophotometer because of its structural design. This may have resulted in a small air gap between the two surfaces that further influenced the optical path of the light that reaches the detector during analysis. This can be visualized in Figure 31.

Figure 31: Evaluation of scanning methodology for the handheld spectrophotometer

Temperature ranged between 24.90-27.30 °C, during the experiment. Table 19, Table 20 and Table 21 shows the different pretreatments that were used for spectra optimization of the benchtop spectrophotometer with optical glass, handheld spectrophotometer with optical glass and handheld spectrophotometer with LDPE plastic bag, respectively.

Table 19: Pretreatment selection for protein powder spectra obtained using the benchtop spectrophotometer

	PLS prediction of urea in all mixtures		LDA classification of all mixtures at 0.5% w/w adulteration	
	R^2CV	RMSECV (% w/w)	Average recognition (%)	Average prediction (%)
Raw	0.85	0.22	99.01	98.77
Savitzky-Golay smoothing (21 points)	0.84	0.23	99.05	98.74
MSC	0.86	0.21	99.23	97.55
Savitzky-Golay smoothing (21 points) + MSC	**0.88**	**0.20**	99.01	98.52
SNV	0.85	0.22	99.23	98.51
Savitzky-Golay smoothing (21 points) + SNV	0.84	0.23	**99.51**	**98.90**
1st Derivative	0.85	0.22	99.38	98.52
1st Derivative+MSC	0.86	0.21	99.13	98.52
2nd derivative	0.84	0.23	98.89	97.79
2nd Derivative +MSC	0.87	0.21	99.25	98.04
Detrend	0.85	0.22	98.27	98.52

Table 20: Pretreatment selection for protein powder spectra obtained using the handheld spectrophotometer and optical glass

	PLS prediction of urea in all mixtures		LDA classification of all mixtures at 0.5% w/w adulteration	
	R^2CV	RMSECV (% w/w)	Average recognition (%)	Average prediction (%)
Raw	0.78	0.27	71.74	49.37
Savitzky-Golay smoothing (21 points)	0.78	0.27	65.56	50.85
MSC	0.79	0.26	75.98	47.15
Savitzky-Golay smoothing (21 points) + MSC	0.79	0.26	65.46	49.86
SNV	0.80	0.26	66.50	46.91
Savitzky-Golay smoothing (21 points) + SNV	**0.80**	**0.26**	**75.94**	**50.84**
1st Derivative	0.78	0.27	69.04	51.08
1st derivative+MSC	0.75	0.29	72.64	49.39
2nd Derivative	0.78	0.27	74.02	50.19
2nd Derivative +MSC	0.79	0.26	58.26	47.87
Detrend	0.78	0.27	70.89	45.20

Table 21: Pretreatment selection for protein powder spectra obtained using the handheld spectrophotometer and LDPE

	PLS prediction of urea in all mixtures		LDA classification of all mixtures at 0.5% w/w adulteration	
	R^2CV	RMSECV (% w/w)	Average recognition (%)	Average prediction (%)
Raw	0.72	0.30	71.68	39.71
Savitzky-Golay smoothing (21 points)	0.71	0.31	78.11	48.47
MSC	0.75	0.29	68.05	40.39
Savitzky-Golay smoothing (21 points) + MSC	0.74	0.30	77.57	47.59
SNV	0.74	0.29	69.70	42.44
Savitzky-Golay smoothing (21 points) + SNV	**0.75**	**0.29**	**79.23**	**49.77**
1st Derivative	0.72	0.30	78.86	46.72
1st Derivative+MSC	0.75	0.29	75.46	46.27
2nd derivative	0.73	0.30	78.24	48.08
2nd Derivative +MSC	0.73	0.30	70.03	47.41
Detrend	0.73	0.30	69.65	39.32

From Table 19, applying the Savitzky-Golay smoothing (21 points) before MSC was the most effective pretreatment for predicting melamine in the protein powder mixtures using PLSR. This was deemed as the optimum pretreatment for PLSR analysis using benchtop spectrophotometer and optical glass. Applying the Savitzky-Golay smoothing (21 points) before SNV gave the highest classification accuracies for classifying all the different mixture combinations (U, G, T, M, GT, UG, GM, UT, TM, UM, UGT, GTM, UGM, UTM, UGTM) and pure whey, beef and pea protein powder. This was deemed as the optimum pretreatment for LDA analysis using benchtop spectrophotometer and optical glass.

Table 20 and Table 21 showed that applying the Savitzky-Golay smoothing (21 points) before SNV was the most effective pretreatment for predicting melamine in the protein powder mixtures using PLSR, when the handheld spectrophotometer was used with both optical glass and LDPE. Applying the Savitzky-Golay smoothing (21 points) before SNV also gave the highest classification accuracies for classifying all the different mixture combinations (U, G, T, M, GT, UG, GM, UT, TM, UM, UGT, GTM, UGM, UTM, UGTM) and pure whey, beef and pea protein powder when the handheld spectrophotometer was used with both optical glass and LDPE. Key parameters associated with the control of spectrum noise are slit width, detector gain, and integration (response) time (Wang and Zhou 2011), so different spectrophotometers may respond to different types of pretreatment for spectral optimization. In the near infrared region (700-2500

nm), food products and their adulterants can be characterized by certain absorption bands that relay important information about their chemical structure (Aouadi et al. 2020) and can be useful for authentication through fingerprinting. The wavelength range 1180-1260 nm has been reported to be related to the second overtone of C—H stretching (Song et al. 2018). The 1450 nm band has been associated with the O—H first overtone and is sensitive to H bonding whereas, the band 1430 nm corresponds to the second and first overtone regions of N—H bonds or first overtone vibration of water (Inagaki, Watanabe, and Tsuchikawa 2017). The band at 1530 nm signifies the presence of either N—H stretching vibration of the amide group from protein to stretching and O—H (Song et al. 2018). The band at 1570 nm corresponds either to N—H stretching vibrations of amide groups or O—H of vibrating water whereas, the band range 1580-1650 nm corresponds to N—H (Osborne 2000). Bands 1600-1650 nm signify the presence of carbonyl groups (C=O) (Rodriguez-Saona et al. 2006).

The chemical structures of the adulterants themselves could be related to some of these important absorption bands in the absorption plot. Melamine for instance, has three nitrogen atoms attached to three amine groups, taurine is characterized by a sulphate group and an amine group, urea is characterized by carbonyl groups (C=O) and two amines, glycine is characterized by a carbonyl group, a hydroxyl group and an amine group. Correlations can be made with the bands give a hint about adulterant presence, their mixture combinations or concentrations in the three protein powder mixtures.

5.5.2. Classification of pure and adulterated protein powders with NIRS based on their protein base

Figure 32, shows the classification plots for adulterated and non-adulterated whey, beef and pea protein powder using the benchtop spectrophotometer and optical glass cuvette (A), handheld spectrophotometer and optical glass cuvette (B) and handheld spectrophotometer with LDPE plastic bag (C). All the protein powders could be visually distinguished irrespective of the adulterant combination or concentration. There was 100% correct classification in both recognition and cross-validation for all the datasets.

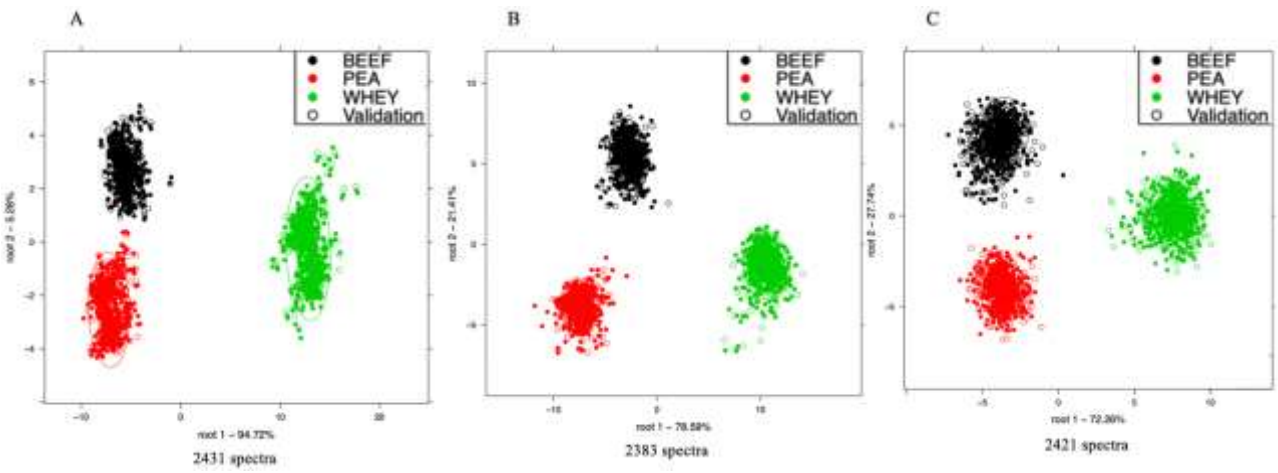

Figure 32: Classification of adulterated and non-adulterated whey, beef and pea protein powder using the benchtop spectrophotometer and optical glass cuvette (A), handheld spectrophotometer and optical glass cuvette (B) and handheld spectrophotometer with LDPE plastic bag (C)

5.5.3. Classification of pure protein powders and their adulterated mixture combinations

Figure 33, shows the plot for the classification of whey, beef and pea protein powder and their adulterated mixture combinations and using the benchtop spectrophotometer and optical glass cuvette (A), handheld spectrophotometer and optical glass cuvette (B) and handheld spectrophotometer with LDPE plastic bag (C). All the plots were characterized by an increasing adulteration (0%-3% w/v) from the center to the extremities. In plot A and B, all the single adulterant mixtures could be separated and even some of the dual and multiple adulterant mixtures could be separated as well. There was average recognition accuracy of 74.01% and prediction accuracy of 74.09% using the benchtop spectrophotometer. Using the handheld spectrophotometer with optical glass yielded average recognition accuracies of 58.99% and prediction of 56.46%. Using the handheld spectrophotometer with LDPE plastic yielded average recognition accuracies of 62.17% and prediction of 54.48%.

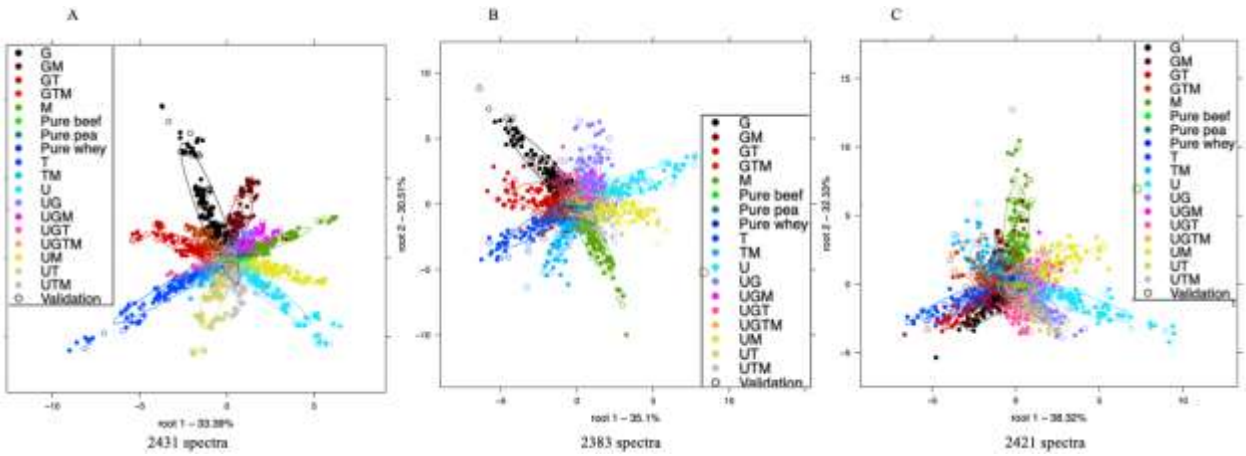

Figure 33: Mixture classification of adulterated and non-adulterated protein powder using the benchtop spectrophotometer and optical glass cuvette (A), handheld spectrophotometer and optical glass cuvette (B) and handheld spectrophotometer with LDPE plastic bag (C)

Figure 33 showed an increasing adulteration from the center of the plot to the extremities, signaling the influence of the different adulterant concentrations on mixture classifications so detailed analysis were performed to evaluate the potential of the instruments in detecting adulteration at the lowest adulterant concentration of 0.5% w/w.

5.5.4. Classification of pure and adulterated protein powder mixtures at the lowest adulterant concentration of 0.5% w/w with NIRS

These models were developed to assume situations where producers may use very low concentrations because practically, tracking adulteration involves determining whether adulteration has occurred or not and not necessary the percentage of its existence. Thus, 0.5% w/w being the lowest adulterant concentration tested in this study, it’s detection regardless of the protein type would be more practical and be of much significance.

Using the benchtop spectrophotometer, there was 99.47% average recognition and 98.75% average prediction for classifying all the protein powders at the lowest adulterant concentration of 0.5% w/w using benchtop spectrophotometer. There was visual separation of all the mixtures in the classification plot (Figure 34). There was no misclassification between pure protein powders and all mixture combinations with cross-validation (Appendices - A7, Table 39).

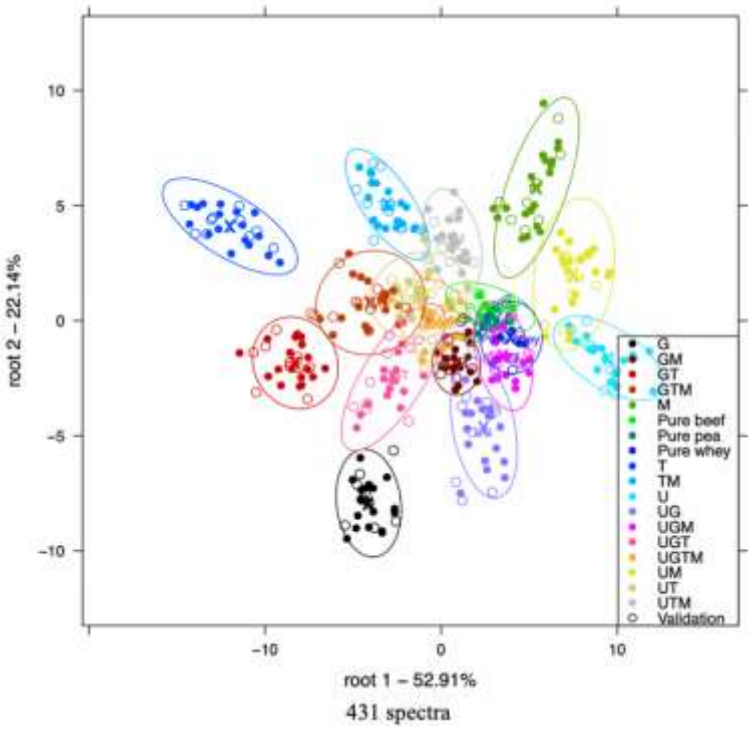

Figure 34: Mixture classification of adulterated and non-adulterated protein powder at lowest adulterant concentration 0.5% w/w using the benchtop spectrophotometer

Using handheld spectrophotometer and optical glass yielded average recognition of 65.13% and average prediction of 53.49%. Pure whey, beef and pea protein powders could be classified with 66.89%, 100%, 89% accuracies respectively. There was 33.11% and 11% misclassification of pure whey and pea protein powder respectively as adulterated protein powder samples (Appendices - A7, Table 41).

Using the handheld spectrometer with LDPE plastic bag yielded average recognition 83.79% and average prediction 56.19% achieved using handheld spectrophotometer with LDPE plastic bag. Pure whey, beef and pea protein powders could be classified with 66.89%, 66.89%, 89% accuracies respectively and misclassifications of 11.04%, 22.08% and 11% respectively as adulterated protein powder samples. Pure whey and pea protein powders also showed misclassifications (11.04%) amongst themselves (Appendices - A7, Table 43).

5.5.4.1. Performance comparison for using benchtop and handheld spectrophotometers to classify adulterated protein powder mixtures at the lowest adulterant concentration (0.5% w/w)

From the classification results of all three-setups (Figure 35), benchtop spectrophotometer gave the best accuracies for classifying urea, glycine, taurine, melamine and their different mixture combinations at the lowest concentration of 0.5% w/w in whey, beef and pea protein powder. Using the handheld spectrophotometer with LPDE plastic bag gave better average accuracies than

using the handheld spectrophotometer with optical glass. These findings are particularly important because hitherto this studies, adulterant concentrations in literature ranged between 1- 5% w/w and no scanning was done through plastic bag. In addition, classification of the complex adulterant mixture combinations reported in this study signals the potential of the spectrophotometers in detecting complex forms of protein powder adulteration. The findings in this study, provides grounds for detecting novel forms of adulteration in whey, beef and pea protein powder. Compared to traditional methodology such as the Dumas method where, a test run by takes from 5 to 10 min depending on sample weighing and combustion, a single scan with NIRS takes less than 1 min. In terms of expenses, the average cost of the Dumas method is about $25 per sample, whereas the NIR test method can be <$5.00 per sample) (Ingle et al. 2016). The discriminatory and classification accuracies achieved with the benchtop and handheld spectrophotometers proves their potential for detecting urea, glycine, taurine and melamine concentrations as low as 0.5% w/w in protein powders and provides advantages from both time and cost perspective. The handheld spectrophotometer provides an extra advantage of expeditious onsite detection of adulterants. Scanning through the optical glass or LDPE plastic both gave accuracies that can be adapted for practical applications.

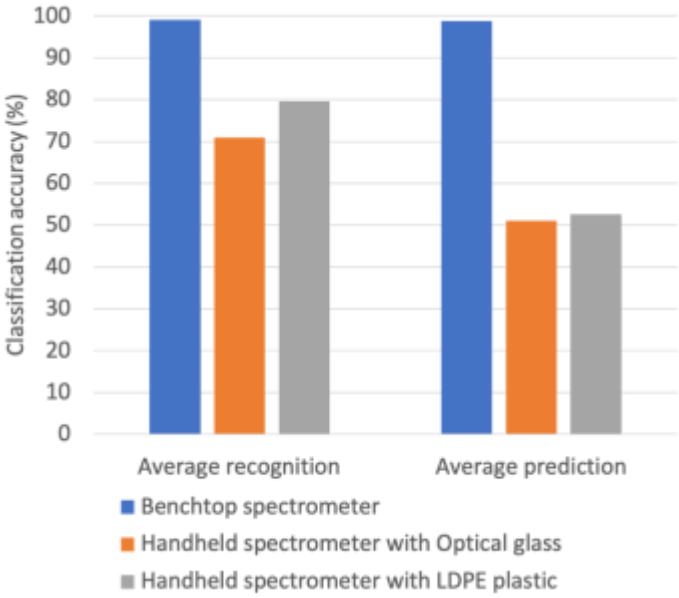

Figure 35: Performance comparison for using benchtop and handheld spectrophotometers to classify adulterated protein powder mixtures at the lowest adulterant concentration (0.5% w/w)

5.5.5. Classification of urea, glycine, taurine and melamine in protein powder using only mixtures with single adulterants

Figure 36, shows the classification plots developed to classify authentic whey (A, B C), beef (D, E, F) and pea (G, H, I) protein powders their mixtures containing only single adulterants

classification plots using benchtop spectrophotometer (A, D, G), handheld spectrophotometer with optical glass (B, E, H) and handheld spectrophotometer with LDPE (C, F, I). Visually, melamine, urea and taurine could be separated from all the other adulterants but there were some overlapping between pure whey, beef or pea protein powder in all cases. In certain cases, glycine could also be visually separated. The best separation pattern of urea, glycine, taurine and melamine was achieved with the benchtop spectrophotometer for all the three protein powders.

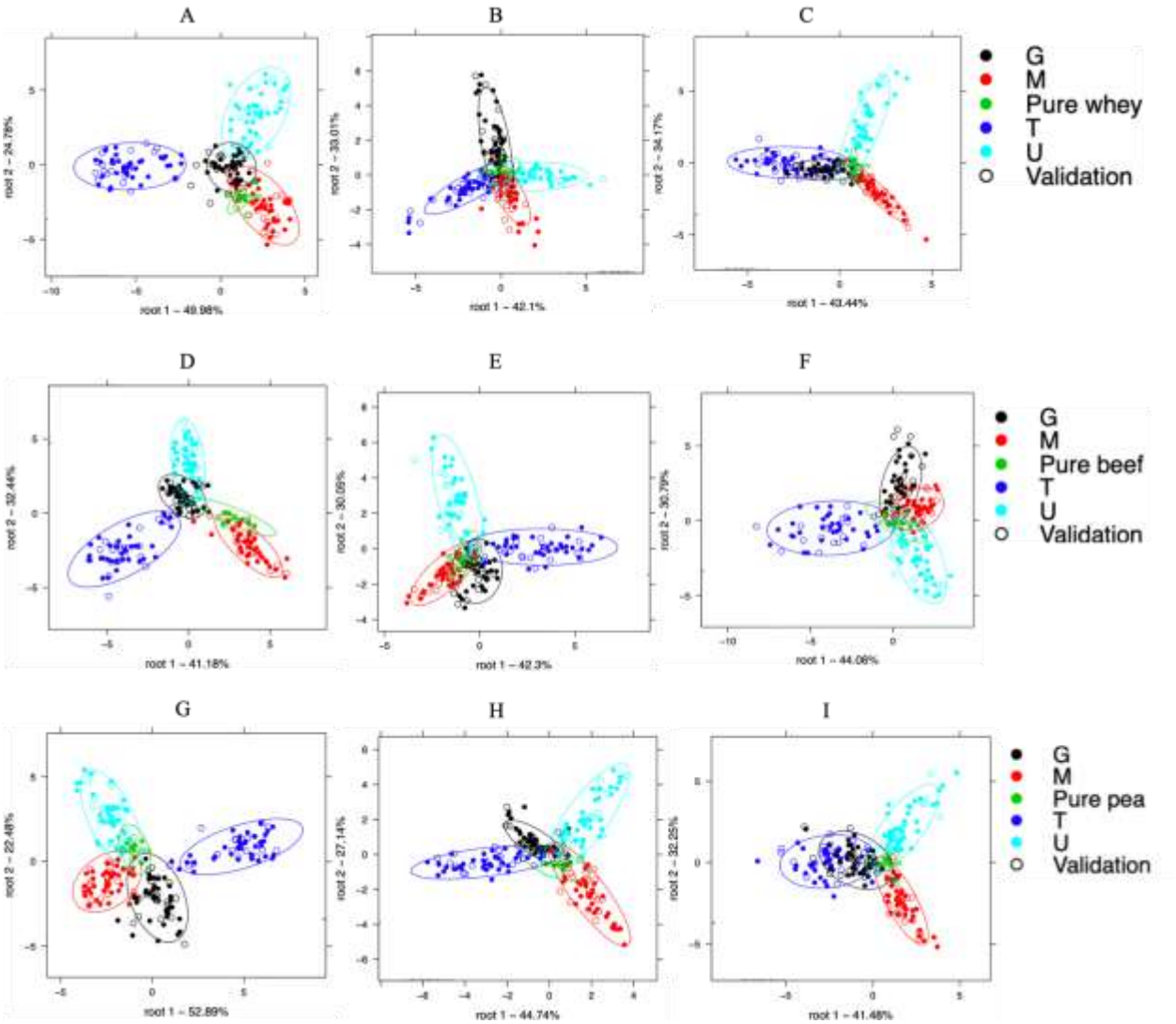

Figure 36: classification plots for whey (A, B C), beef (D, E, F) and pea (G, H, I) protein powder containing only single adulterants between 0.5 -3% w/w. Benchtop spectrophotometer (A, D, G), handheld spectrophotometer with optical glass cuvette (B, E, H) and handheld spectrophotometer with LDPE (C, F, I).

Using the handheld spectrophotometer with optical glass yielded average 91.72% recognition and 90.43% prediction for classification of single adulterants in whey protein powder (Table 22). With cross-validation, only melamine (11%) was misclassified as pure whey protein powder. Using the handheld spectrophotometer with LDPE plastic bag yielded an average

recognition accuracy of 93.35% and 90.03% prediction were achieved when the LDPE plastic bag was used. With cross-validation, only glycine (11%) was misclassified as pure whey protein powder.

Table 22: Confusion matrix for classifying urea (U), glycine (G), taurine (T) and melamine (M) in whey protein powder by scanning through optical glass or LDPE plastic bag with the handheld spectrophotometer. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%)

		Average Recognition (91.72%)					Average Cross-validation (90.43%)				
		U	G	T	M	Pure	U	G	T	M	Pure
Optical glass	U	86.43	0.92	0	0	5.51	82	0	0	0	0
	G	0	87.05	0.97	0	0	0	87.06	0	0	0
	T	9.7	6.47	1.97	99.08	5.51	0	0	94.12	0	0
	M	0	0	97.06	0	0	12	7.39	3.94	100	11
	Pure	3.87	5.56	0	0.92	88.98	6	5.56	1.94	0	89
		Average Recognition (93.35%)					Average Cross-validation (90.03%)				
		U	G	T	M	Pure	U	G	T	M	Pure
LDPE plastic	U	91.18	0	0	0	0	90.23	0	0	0	0
	G	0.97	90.34	6.41	0	0	1.94	87.74	5.66	0	11
	T	0	0	91.74	0	0	0	0	92.47	0	0
	M	1.97	0	0	93.53	0	0	1.74	0	90.72	0
	Pure	5.88	9.66	1.84	6.47	100	7.83	10.53	1.87	9.28	89

For beef protein powder mixtures, there was an average classification accuracy of 95.66% recognition and 94.80% prediction accuracy when handheld spectrophotometer was used with optical glass (Table 23). Average recognition and prediction accuracy were 95.82% and 91.99% respectively when handheld spectrophotometer and LDPE plastic bag was used. With or without cross-validation, pure beef protein powders could be classified with 100% correct accuracy when they were scanned through either optical glass or LDPE plastic bag.

Table 23: Confusion matrix for classifying urea (U), glycine (G), taurine (T) and melamine (M) in beef protein powder by scanning through optical glass or LDPE plastic bag with the handheld spectrophotometer. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%)

		Average Recognition (95.66%)					Average Cross-validation (94.80%)				
		U	G	T	M	Pure	U	G	T	M	Pure
Optical glass	U	95.09	0	0	0	0	96.06	0	0	0	0
	G	0	96.88	3.91	1.95	0	0	93.75	1.94	1.98	0
	T	0	0	93.15	0	0	0	0	92.18	0	0
	M	2.94	0	0	93.19	0	3.94	0	0	92.02	0
	Pure	1.97	3.12	2.94	4.86	100	0	6.25	5.88	6	100
		Average Recognition (95.82%)					Average Cross-validation (91.99%)				
		U	G	T	M	Pure	U	G	T	M	Pure
LDPE plastic	U	95.4	0	0	0	0	90.55	0	0	0	0
	G	0	95.84	0	0	0	0	85.44	0	0	0
	T	0	0	93.75	0	0	0	0	93.75	1.94	0
	M	1.84	0	0	94.12	0	3.79	6.25	0	90.23	0
	Pure	2.75	4.16	6.25	5.88	100	5.66	8.31	6.25	7.83	100

For pea protein powder mixtures, average 94.65% recognition and 93.71% prediction accuracy were achieved when handheld spectrophotometer was used with optical glass (Table 24). With no cross-validation, only samples containing melamine (5.51%) were misclassified as pure pea protein powder. This was the same with cross-validation but with a higher misclassification rate of 11%. Using handheld spectrophotometer and LDPE plastic bag, accuracies of 93.55% (recognition) and 94.02% (prediction) were achieved. With no cross-validation, only samples containing glycine (11.17%) were misclassified as pure pea protein powder. This was the same with cross-validation but with a slightly lower misclassification rate of 11%.

Table 24: Confusion matrix for classifying urea (U), glycine (G), taurine (T) and melamine (M) in pea protein powder by scanning through optical glass or LDPE plastic bag with the handheld spectrophotometer. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%)

		Average Recognition (94.65%)					Average Cross-validation (93.71%)				
		U	G	T	M	Pure	U	G	T	M	Pure
Optical glass	U	92.58	0	0	1.86	0	92.56	0	1.87	1.83	0
	G	0	96.31	3.66	0	0	0	96.28	1.87	0	0
	T	0	0	93.59	0	0	0	0	94.39	0	0
	M	1.86	0	0	96.28	5.5	3.72	0	0	96.33	11
	Pure	5.56	3.69	2.75	1.86	94.5	3.72	3.72	1.87	1.83	89
		Average Recognition (94.08%)					Average Cross-validation (92.59%)				
		U	G	T	M	Pure	U	G	T	M	Pure
LDPE plastic	U	98.17	0	0	0	0	96.33	0	1.83	0	0
	G	0.92	97.22	3.7	0	11.17	1.83	94.44	3.72	0	11
	T	0	0	92.61	0	0	0	0	90.72	0	0
	M	0	0	0.92	93.59	0	0	1.83	0	92.47	0
	Pure	0.92	2.78	2.78	6.41	88.83	1.83	3.72	3.72	7.53	89

The best accuracies for classifying urea, glycine, taurine and melamine in protein powder mixtures with single adulterants were achieved with the benchtop spectrophotometer for all the three protein powders. There was average 98.71% recognition and 96.28% prediction for whey protein powder, 100% recognition and 99.62% prediction for beef protein powder and 98.89% recognition and 98.88% prediction for pea. With cross-validation, pure beef and pea protein powder could be predicted with 100% correct accuracy but 11% of samples containing melamine were misclassified as pure whey protein powder. Table 25 shows a summary of the cross-validated accuracies for pure whey, beef, and pea protein powder mixtures after using the benchtop and

handheld spectrophotometers to classify authentic protein powders and protein powder mixtures containing single adulterants.

Table 25: Cross-validated accuracies for pure whey, beef, and pea protein powder mixtures after using the benchtop and handheld spectrophotometers to classify authentic protein powders and protein powder mixtures containing single adulterants

	Pure protein powder	Correct classification (%)	Misclassification (%)	Misclassified adulterant
Benchtop spectrophotometer with optical glass	Whey	89	11	Melamine
	Beef	100	0	-
	Pea	100	0	-
Handheld spectrophotometer with optical glass	Whey	89	11	Melamine
	Beef	100	0	-
	Pea	89	11	Melamine
Handheld spectrophotometer with LDPE plastic	Whey	89	11	Glycine
	Beef	100	0	-
	Pea	89	11	Glycine

5.5.6. PLSR prediction of urea, glycine, taurine and melamine concentrations in protein powder mixtures

From Table 26, all the models could predict the adulterants with R^2CV in the range of 0.74-0.93 and RMSECV in the range of 0.21- 1.57% w/w of adulterated protein powders. The best models were achieved with the benchtop spectrophotometer but the accuracies achieved with the handheld spectrophotometer signifies its potential for such analysis as well. The models developed with the dataset from scanning through the LDPE plastic bag was generally weaker compared to those developed with the dataset from scanning through optical glass with the handheld spectrophotometer. Urea could be predicted with the highest accuracies for all three setups: benchtop spectrophotometer with optical glass, handheld spectrophotometer with optical glass and handheld spectrophotometer with LDPE plastic. The best model was R^2CV of 0.93 and RMSECV of 0.21% w/w using the benchtop spectrophotometer (Figure 37).Models were also built to classify the urea, glycine, taurine and melamine separately in whey protein powder (Appendices – A7, Table 44-Table 46), beef protein powder (Appendices – A7, Table 47-Table 49), and pea protein powder mixtures (Appendices – A7, Table 50-Table 52).

Table 26: PLSR prediction models developed with the benchtop and handheld spectrophotometer to predict urea, glycine, taurine and melamine concentration in protein powder mixtures regardless of their mixture combination

Spectrophotometer	Predicted adulterant	LV	R^2	RMSEC (% w/w)	R^2CV	RMSECV (% w/w)
Benchtop (2431 spectra)	Urea	22	0.95	0.18	0.93	0.21
	Glycine	8	0.90	0.92	0.87	0.97
	Taurine	25	0.95	0.94	0.93	0.94
	Melamine	13	0.93	0.15	0.93	0.16
Handheld with optical glass (2383 spectra)	Urea	20	0.90	0.25	0.89	0.27
	Glycine	18	0.81	0.88	0.79	0.91
	Taurine	15	0.86	1.25	0.86	1.30
	Melamine	17	0.85	0.22	0.82	0.23
Handheld with LDPE plastic (2424 spectra)	Urea	19	0.92	0.23	0.91	0.25
	Glycine	17	0.75	1.01	0.74	1.09
	Taurine	24	0.82	1.48	0.79	1.57
	Melamine	17	0.76	0.28	0.75	0.29

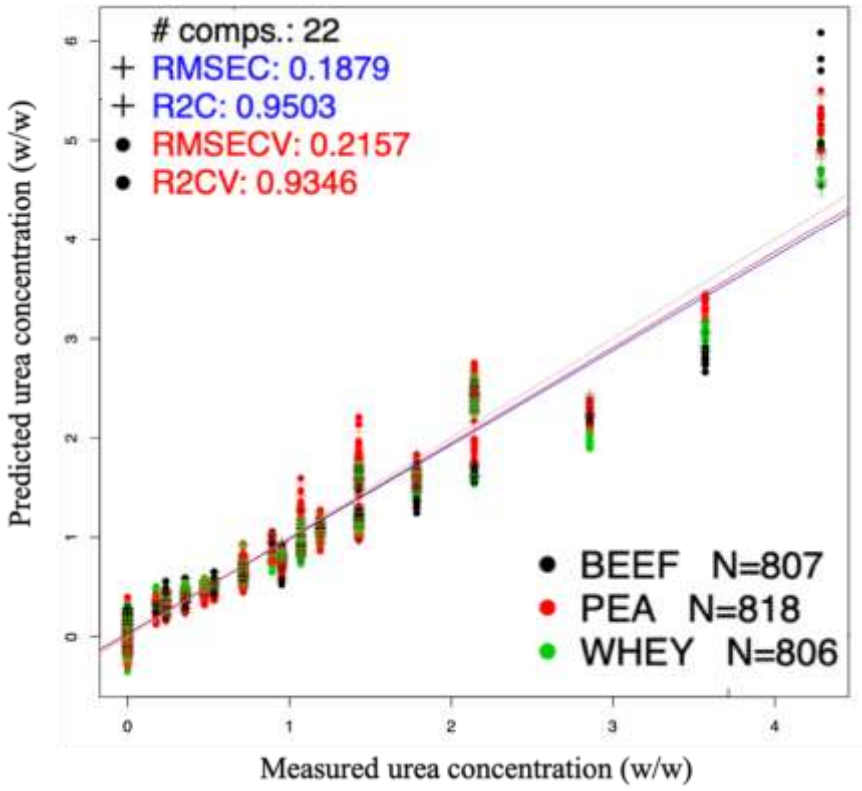

Figure 37: PLS to quantitate urea concentrations in protein powder mixtures at spectral range of 950-1650 nm using the benchtop spectrophotometer

Figure 38 shows the visual performance comparison of the different spectrophotometers for PLS prediction of urea (A), glycine (B), taurine (C) and melamine (D). All the adulterants could be predicted with R^2CV higher than 0.80 irrespective of the scanning medium or spectrophotometer. RMSECV was generally lowest for the prediction of melamine in the protein powder mixtures. Glycine yielded the highest RMSECV.

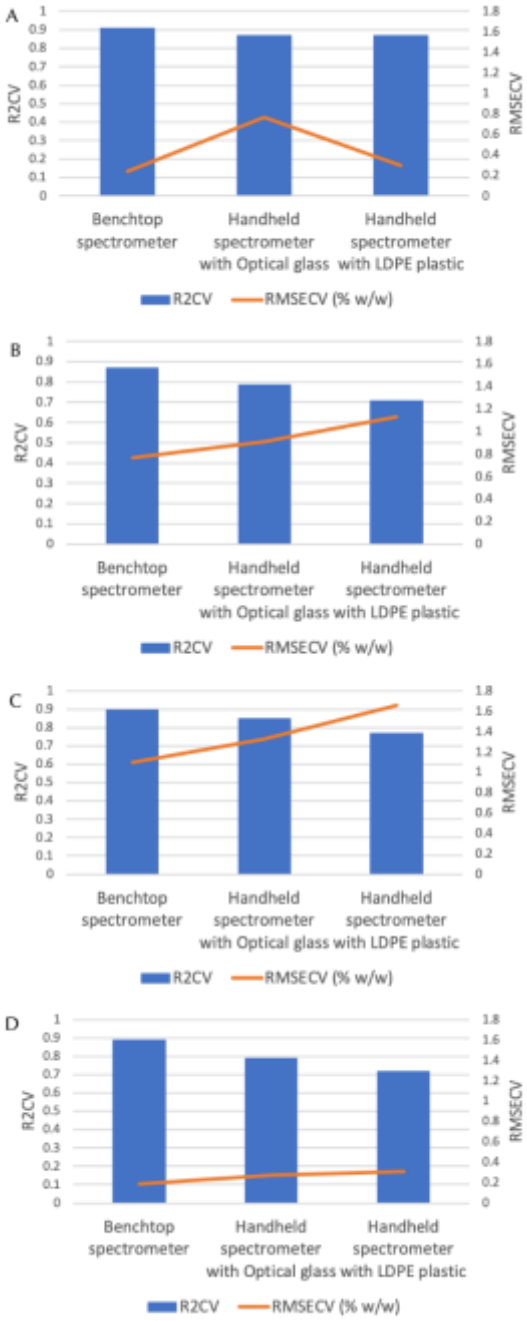

Figure 38: PLS comparative performance of the different spectrophotometers for prediction of urea (A), glycine (B), taurine (C) and melamine (D) in authentic and adulterated whey, beef and pea protein powder mixtures irrespective of their protein base

5.5.7. PLSR prediction of urea, glycine, taurine and melamine concentrations in protein powder mixtures from analysis with independent data

All the models could predict the adulterants with R^2pred in the range 0.72-0.94 and RMSEP in the range 0.18- 1.59% w/w of adulterated protein powders (Table 27). Urea could be predicted with the highest accuracies for all three cases: with benchtop spectrophotometer, handheld spectrophotometer with optical glass and handheld spectrophotometer with LDPE plastic bag, the best was as R^2pred of 0.94 and RMSECV of 0.18% w/w using the benchtop spectrophotometer (Figure 39).

Table 27: Independent PLS regression models developed with the benchtop and handheld spectrophotometer to predict urea, glycine, taurine and melamine in all adulterated protein powder samples at spectral range of 950-1650 nm

Spectrophotometer	Predicted adulterant	LV	R^2pred	RMSEP (% w/w)
Benchtop Cal: 1624 Valid: 807	Urea	22	0.94	0.18
	Glycine	9	0.77	0.94
	Taurine	20	0.93	0.91
	Melamine	11	0.93	0.15
Handheld with optical glass Cal: 1591 Valid: 792	Urea	20	0.90	0.25
	Glycine	17	0.79	0.91
	Taurine	16	0.86	1.29
	Melamine	17	0.85	0.20
handheld with LDPE plastic Cal: 1611 Valid: 813	Urea	19	0.91	0.24
	Glycine	17	0.73	1.03
	Taurine	18	0.79	1.59
	Melamine	16	0.72	0.30

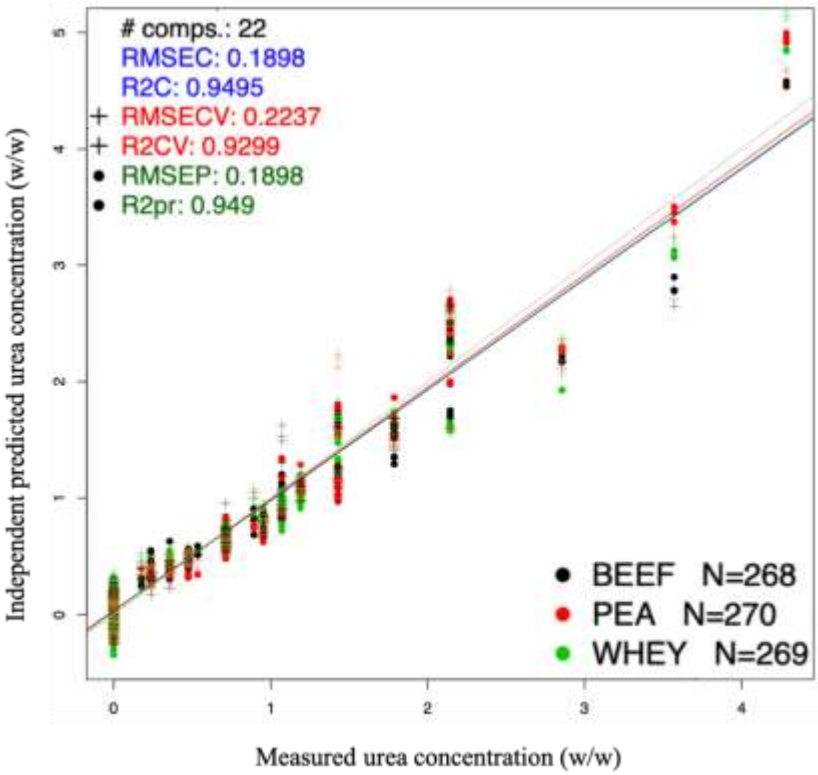

Figure 39: Independent PLS model to quantitate urea concentrations in protein powder mixtures at spectral range of 950-1650 nm using the benchtop spectrophotometer

5.5.8. PLSR prediction of protein powder concentrations in protein powder mixtures from analysis with independent data

Table 28 shows the independent PLS regression models developed with the benchtop and handheld spectrophotometers to predict protein powder concentrations in all samples and also in protein powder mixtures that contained single mixtures of urea, glycine, taurine and melamine at concentrations of 0.5% w/w – 3% w/w.

For prediction of protein powder concentration in all mixtures, using the handheld spectrophotometer with optical glass gave R^2CV 0.84, RMSECV 1.38% w/w, R^2pred 0.84 and RMSEP 1.38% w/w. Using the handheld spectrophotometer with optical LDPE plastic gave R^2CV 0.86, RMSECV 1.39% w/w, R^2pred 0.84 and RMSEP 1.47% w/w. Based on the R^2CV and RMSECV values using the LDPE plastic bag gave the best results for predicting protein powder in the samples when the handheld spectrophotometer was used, compared to using the handheld spectrophotometer with optical glass. They both however, had similar R^2pred and RMSEP values. Using the benchtop spectrophotometer gave the best model for predicting protein powder concentrations in all the samples with R^2CV 0.86, RMSECV 1.36% w/w, R^2pred 0.87 and RMSEP 1.30% w/w.

Table 28: Independent PLS regression models developed with the benchtop and handheld spectrophotometer to predict protein powder concentrations in all samples and also in protein powder mixtures that contained single mixtures of urea, glycine, taurine and melamine at concentrations of 0.5% w/w – 3% w/w at spectral range of 950-1650 nm

	Spectrophotometer	LV	R^2CV	RMSECV (% w/w)	R^2pred	RMSEP (% w/w)
Whole dataset	Benchtop spectrophotometer	15	0.86	1.36	0.87	1.30
	Handheld spectrophotometer with optical glass	15	0.84	1.38	0.84	1.38
	Handheld spectrophotometer with LDPE plastic bag	15	0.86	1.39	0.84	1.47
Single adulterants	Benchtop spectrophotometer	15	0.90	1.41	0.93	1.22
	Handheld spectrophotometer with optical glass	15	0.84	1.18	0.88	1.58
	Handheld spectrophotometer with LDPE plastic bag	15	0.91	1.36	0.92	1.33

For prediction of protein powder concentrations in protein powder mixtures that contained single mixtures of urea, glycine, taurine and melamine at concentrations of 0.5% w/w – 3% w/w (Table 28), using the handheld spectrophotometer with optical glass gave R^2CV 0.84, RMSECV 1.18% w/w, R^2pred 0.88 and RMSEP 1.58% w/w. Using the handheld spectrophotometer with optical LDPE plastic gave R^2CV 0.91, RMSECV 1.36% w/w, R^2pred 0.92 and RMSEP 1.33% w/w. Based on the R^2CV, RMSECV, R^2pred and RMSEP values, using the handheld spectrophotometer with LDPE plastic bag gave the best results compared to using the handheld spectrophotometer with optical glass for predicting protein powder concentrations in protein powder mixtures that contained single mixtures of urea, glycine, taurine and melamine at concentrations of 0.5– 3% w/w. Using the benchtop spectrophotometer gave the best model for predicting protein powder concentrations in protein powder mixtures that contained single mixtures of urea, glycine, taurine and melamine at concentrations of 0.5% w/w – 3% w/w. There was R^2CV 0.90, RMSECV 1.41% w/w, R^2pred 0.93 and RMSEP 1.22% w/w.

5.5.9. LODmin, LODmax, LOQmin and LOQmax for the determination of urea, glycine, taurine and melamine in protein powder

Table 29, shows the LODmin, LODmax, LOQmin and LOQmax and the explained variance between the X (actual concentrations) and Y (predicted concentrations) variables in the dataset. Typically, LOQ will be found at the same or higher concentration than LOD, but how much higher depends on the specifications for bias and imprecision used to define it (Armbruster and Pry 2008), as it is dependent on LOD.

Table 29: Model validation for the prediction of urea, glycine, taurine and melamine in whey, beef, and pea protein powder mixtures

	Adulterant	LV	LODmin (%)	LODmax (%)	LOQmin (%)	LOQmax (%)	E_varX (%)	E_varY (%)
Benchtop	Urea	22	0.11	0.26	0.34	0.79	100.00	100.00
	Glycine	11	0.15	0.86	0.44	2.59	100.00	99.99
	Taurine	18	0.44	1.08	1.32	3.23	100.00	100.00
	Melamine	11	0.07	0.19	0.22	0.56	100.00	100.00
Handheld with optical glass	Urea	20	0.18	0.86	0.53	2.58	100.00	100.00
	Glycine	18	0.39	2.31	1.17	6.92	100.00	99.99
	Taurine	17	0.53	3.10	1.59	9.29	100.00	99.99
	Melamine	17	0.19	0.61	0.56	1.83	100.00	99.99
Handheld with LDPE plastic	Urea	19	0.20	0.34	0.60	1.01	100.00	100.00
	Glycine	17	0.74	1.30	2.23	3.90	100.00	99.99
	Taurine	26	0.85	1.99	2.56	5.97	100.00	99.99
	Melamine	17	0.16	0.32	0.47	0.97	100.00	99.99

LV: latent variable

LODmin: Limit of detection minimum value (% w/w)

LODmax: Limit of detection maximum value (% w/w)

LOQmin: Limit of quantification minimum value (% w/w)

LODmax: Limit of quantification maximum value (% w/w)

E_varX: Explained variance X

Handheld spectrophotometer with optical glass had LODmin in the range 0.18 -0.53% w/w. Only taurine had LODmin higher than the minimum adulterant concentration of 0.5% w/w used in this study. LODmax was in the range 0.61-3.10% w/w. Only taurine had LODmax higher than the maximum adulterant concentration of 3% w/w used in this study.

Handheld spectrophotometer with LDPE plastic had LODmin in the range 0.16 - 0.85%w/w. Only taurine and glycine had LODmin higher than the minimum adulterant concentration of 0.5% w/w used in this study. LODmax was in the range 0.32-1.99% w/w, which was below the maximum adulterant concentration of 3% w/w used in this study.

Benchtop spectrophotometer gave the lowest LOD min/max values for the determination of urea, glycine, taurine and melamine in the protein powder mixtures. The LODmin using this instrument were in the range 0.11-0.44% w/w, which was below the tested minimum adulterant concentration of 0.5% w/w for all the adulterants. LODmax was in the range of 0.56-3.23% w/w. Only taurine had LODmax higher than the maximum adulterant concentration of 3% w/w used in this study when the benchtop spectrophotometer was used.

According to the International Union of Pure and Applied Chemistry (IUPAC), LODmin/max measurements are recommended because they bring together two important analytical concepts: the sensitivity and the precision in the analytical determinations (Allegrini and Olivieri 2014). Differences in explained variances between the X (actual concentrations) and Y (predicted concentrations) were between 0-0.01%. Comparatively, using the handheld spectrophotometer and optical glass yielded lower LODmin/max and LOQmin/max for some of the adulterants than when the handheld spectrophotometer was used with optical glass. Samples scanned through the LDPE plastic bag, however, had a better repeatability when average LOD and LOQ's were evaluated (Figure 40). Urea and melamine had the lowest LOD and LOQ irrespective of the instrument or scanning method. Taurine always had the highest LOD and LOQ irrespective of the instrument or scanning method. Lukacs *et al.* (2018) also reported LOD and LOQ values within this range for determination of urea, taurine and histidine in whey protein powder.

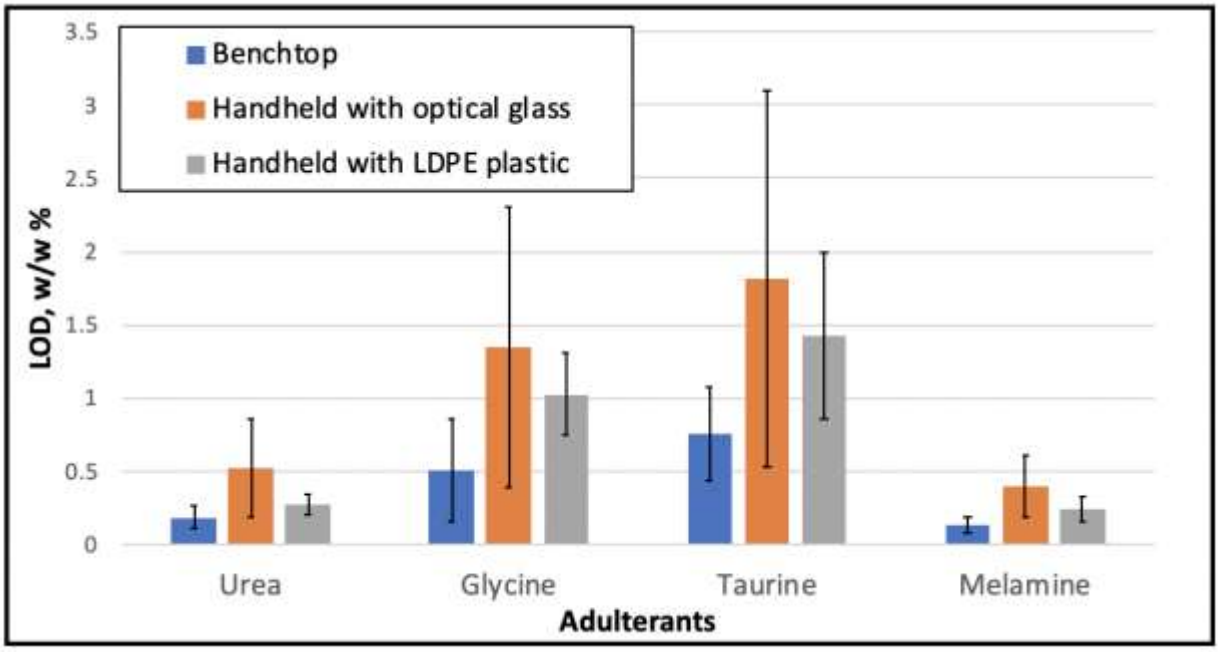

Figure 40: Average limit of detection (LODmin/max) and standard deviation for the different adulterants using the benchtop spectrophotometer, handheld spectrophotometer with optical glass and handheld spectrophotometer with LDPE plastic

5.6. Challenges with the handheld spectrophotometer

According to the manual of the handheld spectrophotometer used in this study, the NR-S-G-1(InnoSpectra 2000), the collection lens gathers light from a 2.5 mm diameter region at the sample window. The size of the collection region is matched to the nominal illumination spot size created by the lens-end lamps. This requires that the sample be placed directly against the sapphire window, where the two angled light source paths intersect the collection vision cone of the lens. If the sample is shifted farther away from the window, the sample may not receive enough illumination for the system to perform an accurate scan (Figure 41). The optical path of the polychromatic and monochromatic light thus, is always important and influences the amount of light that gets back to the detector. This can also be influenced by factors such as the layer thickness of the scanning medium, the experimental setup and the instrument design so all these need to be carefully considered before experiments with similar spectrophotometers.

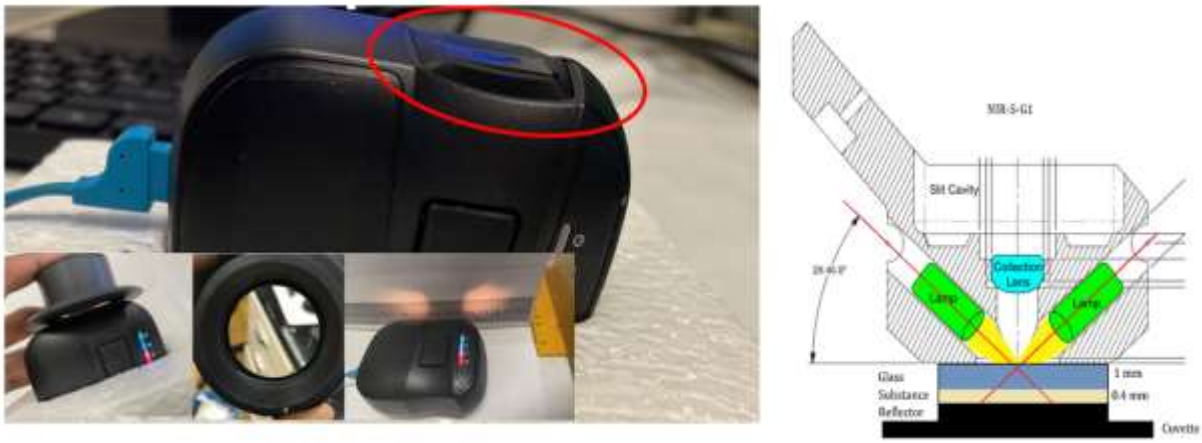

Figure 41: Challenges of using handheld spectrophotometer with optical glass

6. CONCLUSION AND RECOMMENDATION

When authentic wines (in increasing order of quality): Tokaji Forditas II, Tokaji Forditas I, Tokaji Aszu II and Tokaji Aszu I were acquired from the Tokaji region of Hungary and analysed with the benchtop and handheld spectrophotometers, all the authentic wines could be classified (linear discriminant analysis) with 100% accuracy using the benchtop spectrophotometer at a wavelength range of 950-1650 nm. Handheld spectrophotometer could only classify authentic Tokaji Forditas I, Forditas II and Aszu II with 100% accuracy using the wavelength 950-1400 nm. The authentic wines could also, be predicted in Partial least squares regression with R^2CV of 0.92 and an RMSECV of 16.80 g/L of wine using the benchtop spectrophotometer at wavelength 950-1650 nm. Handheld spectrophotometer produced unsatisfactory results for predicting sugar concentrations of the wines. Electronic tongue (e-tongue) analysis after 50% v/v aqua dilution showed that all authentic wines could be classified with 100% correct accuracy. They could also be predicted in PLS with R^2CV of 0.90 and an RMSECV of 17.67 g/L. When authentic Tokaji Forditas I wine and Forditas II wine were adulterated with grape must concentrate and sucrose in different steps to mimic the sugar concentrations of the authentic wines, benchtop spectrophotometer could correctly classify the different Forditas I wine mixtures with average cross-validation accuracies of 96.78% and 98.78% for Forditas II wine mixtures. Handheld spectrophotometer could also, correctly classify adulterated Forditas I and Forditas II wine mixtures with average cross-validation accuracy of 68.22% and 76.06% respectively. Forditas I and Forditas II wine mixtures could also be classified with average cross-validation accuracies of 98.17% and 93.10% using the e-tongue. The results in this study signal a potential for using electronic-tongue and near infrared spectroscopy to track Tokaji wine adulteration. For practically on industrial basis, further studies may be required with higher sample numbers for more robust models. The setup of the handheld spectrophotometer used in this study should also be carefully considered and adapted to better experimental procedures if it is to be used to track Tokaji wine adulteration.

Among the three tested dilution factors for the determination of optimum dilution for e-tongue analysis, 1% w/v dilution produced the best PLSR accuracies for predicting the different poultry mixtures with an R^2CV of 0.95 and RMSECV of 0.80% w/w. Using the optimum dilution factor of 1% w/v for the three tested meat extraction methods, the cooked meat extraction method produced the best results for classifying 100%, 99%, 97%, 95%, 90% and 80% w/w of both poultry and red meat mixtures (beef/pork). There was average recognition of 78.13% and average prediction of 64.72% for classification of poultry mixtures and average recognition of 89.62% and average prediction of 68.77% for classification of red meat mixtures. The cooked meat extraction

method also gave the best PLSR accuracies for predicting red meat mixtures with an R2CV of 0.72 and RMSECV of 3.83% w/w but gave the worst accuracies for predicting poultry mixtures. Sensors HA, BB, ZZ, GA and JB were the most important sensors in discriminating the adulterated meat mixtures. The determined optimal dilution and extraction method can be explored for rapid meat quality control checks with the electronic tongue, however, the study is recommended to be extended with alternative set of meat mixtures, wide range of mixture concentrations to ascertain the reliability of the methods for all meat types. This may also help understand why the cooked meat extraction method worked better for PLSR prediction of beef mixtures compared to the poultry mixtures.

At the lowest adulterant concentration of 0.5% w/w and irrespective of the protein base, benchtop spectrophotometer could classify all the different adulterant mixtures with accuracies of 99.47% average recognition and 98.75% average prediction. Classification results using the handheld spectrophotometer yielded accuracies of 65.13% average recognition and 53.49% average prediction for the optical glass cuvette and average recognition 83.79% and average prediction 56.19% for the LDPE plastic bag. Benchtop spectrophotometer gave the best LDA accuracies for classifying single, dual, triple and quadruple mixtures of urea, glycine, taurine and melamine and that lowest concentration. of 0.5% w/w. Using the handheld spectrophotometer with LPDE plastic bag gave better accuracies than using the handheld spectrophotometer with optical glass. Using the benchtop spectrophotometer gave the best model for predicting protein powder concentrations in all the samples with R^2CV 0.86, RMSECV 1.36% w/w, R^2pred 0.87 and RMSEP 1.30% w/w. Using the handheld spectrophotometer with optical glass gave R^2CV 0.84, RMSECV 1.38% w/w, R^2pred 0.84 and RMSEP 1.38% w/w, for predicting protein powder concentrations in all the samples. Using the handheld spectrophotometer with optical LDPE plastic gave R^2CV 0.86, RMSECV 1.39% w/w, R^2pred 0.84 and RMSEP 1.47% w/w for predicting protein powder concentrations in all the samples. Based on the R^2CV and RMSECV values using the LDPE plastic bag gave the results for predicting protein powder in the samples compared to using the handheld spectrophotometer with optical glass. They both however, had similar R^2pred and RMSEP values. Using the benchtop spectrophotometer gave the best model for predicting protein powder concentrations in protein powder mixtures that contained only single adulterants of urea, glycine, taurine and melamine at concentrations of 0.5 – 3% w/w. There was R^2CV 0.90, RMSECV 1.41% w/w, R^2pred 0.93 and RMSEP 1.22% w/w. Using the handheld spectrophotometer with optical glass gave R^2CV 0.84, RMSECV 1.18% w/w, R^2pred 0.88 and RMSEP 1.58% w/w for predicting protein powder concentrations in protein powder mixtures that contained single mixtures of urea, glycine, taurine and melamine at concentrations of 0.5–3% w/w. Using the handheld spectrophotometer with optical LDPE plastic gave R^2CV 0.91, RMSECV 1.36 w/w, R^2pred 0.92

and RMSEP 1.33% w/w for predicting protein powder concentrations in protein powder mixtures that contained single mixtures of urea, glycine, taurine and melamine at concentrations of 0.5 – 3% w/w. Based on the R^2CV, RMSECV , R^2pred and RMSEP values, using the LDPE plastic bag gave the results compared to using the handheld spectrophotometer with optical glass for predicting protein powder concentrations in protein powder mixtures that contained single mixtures of urea, glycine, taurine and melamine at concentrations of 0.5 – 3% w/w.

Benchtop spectrophotometer yielded the lowest limit of detections (LOD's) and limit of quantifications (LOQ's) for quantifying urea, glycine, taurine and melamine in whey, beef and beef protein powder compared to when the handheld spectrophotometer was used. Comparatively, using the handheld spectrophotometer and optical glass yielded lower LODmin/max and LOQmin/max for some of the adulterants than when the handheld spectrophotometer was used with optical glass was used but samples scanned through the LDPE plastic bag, had a better repeatability when average LOD and LOQ's were evaluated. Urea and melamine had the lowest LOD and LOQ irrespective of the instrument or scanning method. Taurine always had the highest LOD and LOQ irrespective of the instrument or scanning method. For future studies regarding protein powder adulteration, it is recommended to examine practically worthy levels of protein powder adulteration so that specific models may be developed for them with well-defined target accuracies. Assessing the factors that can influence the optical of the handheld spectrometer is very much recommended before starting experiments to obtain reliable experimental datasets.

7. NEW SCIENTIFIC RESULTS

For purposes of these new scientific findings, benchtop spectrophotometer refers to the MetriNIR (MetriNIR, Research Development and Service Co., Budapest, Hungary) whereas, handheld spectrophotometer refers to the NIR-S-G1 (InnoSpectra Co., Hsinchu, Taiwan). E-tongue refers to the Alpha Astree potentiometric electronic tongue (AlphaM.O.S, Toulouse, France) equipped with seven sensors developed for food application (BB, HA, ZZ, GA CA, JE, JB), a reference electrode and a 16 position auto sampler.

1. Authentic Tokaji Forditas II, Forditas I, Aszu II and Aszu I wines were scanned in transflectance mode with benchtop spectrophotometer (950-1650 nm) and handheld spectrophotometer (950-1400 nm) using a glass cuvette with layer thickness 0.4 mm. E-tongue was also used to analyze the wines after 50% v/v aqua dilution. Classification models with linear discriminant analysis (LDA) and prediction models with partial least squares regression (PLSR) were developed using data from all the instruments.
   - **Benchtop spectrophotometer** could classify all the authentic wines with 100% accuracy. Sugar content of authentic wines was predicted with R^2CV of 0.92 and RMSECV of 16.80 g/L of wine.
   - **Handheld spectrophotometer** could classify the authentic wines with average cross-validation accuracy of 94.48% with Forditas I (11.04%) and Aszu II (11.04%) being misclassified as Aszu I. Model developed to predict sugar content of authentic wines with the current setup of handheld spectrophotometer was unsatisfactory.
   - **E-tongue could** classify all authentic wines with 100% correct accuracy and predict the sugar content of authentic wines with R^2CV of 0.90 and RMSECV of 17.67 g/L of wine.

2. Authentic Tokaji wines (Forditas II, Forditas I, Aszu II, Aszu I), adulterated Forditas I and Forditas II wines (add must concentrate to mimic authentic wine sugar concentration) and wine with added sucrose before refermentation were analyzed with spectrophotometers and e-tongue (after 50% v/v aqua dilution). The samples were scanned in transflectance mode with benchtop spectrophotometer (950-1650 nm) and handheld spectrophotometer (950-1400 nm) using a glass cuvette with layer thickness 0.4 mm. Classification models with linear discriminant analysis (LDA) and prediction models with partial least squares regression (PLSR) were developed using data from all the instruments.
   - **Benchtop spectrophotometer** could classify the different Forditas I and Forditas II wine mixtures with average cross-validation accuracies of 96.78% and 98.78%, respectively. There was no misclassification between authentic and adulterated wines.

- **Handheld spectrophotometer** could classify adulterated Forditas I and Forditas II wine mixtures with average cross-validation accuracy of 68.22% and 76.06%, respectively. There were misclassifications between authentic and adulterated wines in both Forditas I and Forditas II wine mixtures.
- **E-tongue** could classify adulterated Forditas I and Forditas II wine mixtures with average cross-validation accuracies of 98.17% and 93.10%. There was no misclassification between authentic and adulterated wines in Forditas I wine mixtures. The wine containing sucrose before refermentation was the only adulterated wine misclassified (53.81%) as an authentic Tokaji wine (Forditas II wine).

3. Raw meat extracts from 100%, 97%, 95%, 90% w/w turkey/chicken mixtures were obtained using aqua dilution levels of 0.5%, 1% and 2% w/v and analyzed using e-tongue. Classification models with linear discriminant analysis (LDA) and prediction models with partial least squares regression (PLSR) were developed using data from e-tongue.
   - **E-tongue** could classify turkey/chicken mixtures after 0.5%, 1% and 2% w/v dilution and predict turkey concentration with R^2CV 0.65, 0.95, 0.81 and RMSECV 2.14% w/w, 0.80% w/w 1.57% w/w for the respective dilution levels. Dilution level (1% w/v) was the optimum among the three tested dilution levels for e-tongue analysis.

4. Raw meat extracts from 100%, 99%, 97%, 95%, 90% and 80% w/w poultry (turkey/chicken) and 100%, 99%, 97%, 95%, 90% and 80% w/w red meat (beef/pork) were obtained using raw meat/frozen meat/cooked extraction method and diluted to 1% w/v. Classification models with linear discriminant analysis (LDA) and prediction models with partial least squares regression (PLSR) were developed using data from e-tongue.
   - **E-tongue** could classify **poultry mixtures** with cross-validation of 58.35%, 62.55%, 64.72% for raw meat, frozen meat, cooked meat extraction methods respectively, and predict them with R^2CV 0.76, 0.81, 0.47 and RMSECV 3.34% w/w, 2.89% w/w and 4.93% w/w respectively.
   - **E-tongue** could classify **red meat mixtures** with cross-validation of 54.25%, 56.41%, 68.77% for raw meat, frozen meat, cooked meat extraction methods respectively, and predict them with R^2CV 0.76, 0.81, 0.47 and RMSECV of 3.34% w/w, 2.89% w/w and 4.93% w/w, respectively.

Whey, beef and pea protein powders were adulterated with urea, glycine, taurine and melamine at a total of 0.5%, 1%, 1.5%, 2%, 2.5% and 3% w/w adulteration using either single, dual, triple or quadruple mixture combinations (16 mixtures). The mixtures were scanned in diffuse reflectance mode using three setups: benchtop spectrophotometer with optical glass, handheld spectrophotometer with optical glass and handheld spectrophotometer with low density polyethylene (LDPE) plastic bag. Wavelength range for all three setups was 950-1650 nm.

5. *When only protein powder mixtures containing single adulterants were analyzed using linear discriminant analysis and predictions with independent data was performed using partial least squares regression:*
   - **All three setups** could classify pure beef protein powder with 100% cross-validation accuracy.
   - **Benchtop spectrophotometer with optical glass** could classify pure whey protein powder with 89% cross-validation accuracy and 11% misclassification as protein powder samples containing melamine. Protein powder concentration in the mixtures could be predicted with R^2CV 0.86, RMSECV 1.36% w/w, R^2pred 0.87 and RMSEP 1.30% w/w respectively.
   - **Handheld spectrophotometer with optical glass** could classify pure whey and pure pea protein powder with 89% cross-validation accuracy each and 11% misclassification each as protein powder samples containing melamine. Protein powder concentration in the mixtures could be predicted with R^2CV 0.84, RMSECV 1.38% w/w, R^2pred 0.84 and RMSEP 1.38% w/w respectively.
   - **Handheld spectrophotometer with LDPE plastic bag** could classify pure whey and pure pea protein powder with 89% cross-validation accuracy each and 11% misclassification each as protein powder samples containing glycine. Protein powder concentration in the mixtures could be predicted with R^2CV 0.86, RMSECV 1.39% w/w, R^2pred 0.84 and RMSEP 1.47% w/w respectively.

6. *When only samples containing the lowest adulterant concentration of 0.5%w/w were analyzed using linear discriminant analysis:*
   - **Benchtop spectrophotometer with optical glass** could classify all the different mixture combinations with average cross-validation accuracy of 98.75%. There was no misclassification between pure protein powders and all mixture combinations.
   - **Handheld spectrophotometer with optical glass** could classify all the different mixture combinations with average cross-validation accuracy of 53.49%. Pure whey, beef and pea protein powders could be classified with 66.89%, 100%, 89% accuracies respectively. There was 33.11% and 11% misclassification of pure whey and pea protein powder respectively as adulterated protein powder samples.

- **Handheld spectrophotometer with LDPE plastic bag** could classify all the different mixture combinations with average cross-validation accuracy of 56.19%. Pure whey, beef and pea protein powders could be classified with 66.89%, 66.89%, 89% accuracies respectively and misclassifications of 11.04%, 22.08% and 11% respectively as adulterated protein powder samples. Pure whey and pea protein powders showed misclassifications (11.04%) amongst themselves.

7. *When all mixtures were analyzed using partial least squares regression with leave-one-sample-out cross-validation:*
   - **Benchtop spectrophotometer with optical glass** could predict urea, glycine, taurine and melamine concentrations with R^2CV 0.93, 0.87, 0.93, 0.93 and RMSECV 0.21, 0.97, 0.94, 0.16% w/w respectively.
   - **Handheld spectrophotometer with optical glass** could predict urea, glycine, taurine and melamine concentrations with R^2CV 0.89, 0.79, 0.86, 0.82 and RMSECV 0.27, 0.91, 1.30, 0.23% w/w respectively.
   - **Handheld spectrophotometer with LDPE plastic bag** could predict urea, glycine, taurine and melamine concentrations with R^2CV 0.91, 0.74, 0.79, 0.75 and RMSECV 0.25, 1.09, 1.57, 0.29% w/w respectively.
   - **All three setups** could predict urea with the highest accuracy.

8. *When limit of detection was calculated for urea, glycine, taurine and melamine in all the mixtures:*
   - **Benchtop spectrophotometer with optical glass** produced the lowest average limit of detections (LOD's) 0.18%, 0.50%, 0.76% and 0.13% for urea, glycine, taurine and melamine respectively. Urea and melamine had average LOD's below the minimum tested adulterant concentration of 0.5% w/w.
   - **Handheld spectrophotometer with optical glass** produced average limit of detections (LOD's) of 0.52%, 1.35, 1.81% and 0.40% for urea, glycine, taurine and melamine respectively. Melamine had average LOD below the minimum tested adulterant concentration of 0.5% w/w.
   - **Handheld spectrophotometer with LDPE plastic bag** produced average limit of detections (LOD's) of 0.27%, 1.02%, 1.42% and 0.24% for urea, glycine, taurine and melamine respectively. Urea and melamine had LOD's below the minimum tested adulterant concentration of 0.5% w/w.
   - **All three setups** produced LOD's in urea, taurine, glycine and melamine that were below the maximum tested adulterant concentration of 3% w/w. Taurine always had the highest LOD.

8. SUMMARY

The great variability in food processing methods is a challenge for food quality control as food quality can be tampered with at any point of the production process. This is particularly a big issue for foods such wine, mean and protein powders with high economic value. This imposes great pressure on the industry to explore new emerging technologies for quality control. As many of the existing conventional methods, are tedious, invasive, expensive, time consuming and require high technical expertise. Advance methods such as near infrared spectroscopy (NIRS) and electronic tongue (e-tongue) are steadily gains grounds for their rapid, affordable, portable, easy to use and fingerprinting capabilities that can be used to track food adulteration. The goal of this thesis was to develop rapid techniques for the determination and prediction of foods with economical and global value using NIRS and e-tongue. The foods under focus were Tokaji wine, poultry (turkey and chicken), red meat (beef and pork) and protein powder concentrates. Linear discriminant analysis (LDA) was used to develop classification models to detect different forms of adulteration these foods while partial least squares regression (PLSR) was used to predict the adulterations.

When authentic wines (in increasing order of quality): Tokaji Forditas II, Tokaji Forditas I, Tokaji Aszu II and Tokaji Aszu I were acquired from the Tokaji region of Hungary and analysed with the benchtop and handheld spectrophotometer, all the authentic wines could be classified (linear discriminant analysis) with 100% accuracy using the benchtop spectrophotometer at a wavelength range of 950-1650 nm. Handheld spectrophotometer could only classify authentic Tokaji Forditas I, Forditas II and Aszu II with 100% accuracy using the wavelength 950-1400 nm. The authentic wines could also, be predicted in Partial least squares regression with R^2CV of 0.92 and an RMSECV of 16.80 g/L of wine using the benchtop spectrophotometer at wavelength 950-1650 nm. Handheld spectrophotometer produced unsatisfactory results for predicting sugar concentrations of the wines. Electronic tongue (e-tongue) analysis after 50% v/v aqua dilution showed that all authentic wines could be classified with 100% correct accuracy. They could also be predicted in PLS with R^2CV of 0.90 and an RMSECV of 17.67 g/L. When authentic Tokaji Forditas I wine and Forditas II wine were adulterated with grape must concentrate and sucrose in different steps to mimic the sugar concentrations of the authentic wines, benchtop spectrophotometer could correctly classify the different Forditas I wine mixtures with average cross-validation accuracies of 96.78% and 98.78% for Forditas II wine mixtures. Handheld spectrophotometer could also, correctly classify adulterated Forditas I and Forditas II wine mixtures with average cross-validation accuracy of 68.22% and 76.06% respectively. Forditas I and Forditas II wine mixtures could also be classified with average cross-validation accuracies of

98.17% and 93.10% using the e-tongue. The results in this study signal a potential for using electronic-tongue and near infrared spectroscopy to track Tokaji wine adulteration. For practically on industrial basis, further may be required with higher sample numbers for more robust models. The setup of the handheld spectrophotometer used in this study should also be carefully considered and adapted to better experimental procedures if it is to be used to track Tokaji wine adulteration.

Among the three tested dilution factors for the determination of optimum dilution for e-tongue analysis, 1% w/v dilution found to produce the best LDA classification results of 100% accuracy for the discrimination of 100%, 97%, 95% and 90% w/w of poultry (turkey/chicken). It also produced the best PLSR accuracies for predicting the different poultry mixtures with an R^2CV of 0.95 and RMSECV of 0.80% w/w. Using the optimum dilution factor of 1% w/v for the three tested meat extraction methods, the cooked meat extraction method produced the best results for classifying 100%, 99%, 97%, 95%, 90% and 80% w/w of both poultry and red meat mixtures (beef/pork). There was average recognition of 78.13% and average prediction 64.72% for classification of poultry mixtures and average recognition of 89.62% and average prediction 68.77% for classification of red meat mixtures. The cooked meat extraction method also gave the best PLSR accuracies for predicting red meat mixtures with an R^2CV of 0.72 and RMSECV of 3.83% w/w but gave the worst accuracies for predicting poultry mixtures. Sensors HA, BB, ZZ, GA and JB were the most important sensors in discriminating the adulterated meat mixtures. The determined optimal dilution and extraction method can be explored for rapid meat quality control checks with the electronic tongue, however, the study is recommended to be extended with alternative set of meat mixtures, wide range of mixture concentrations to ascertain the reliability of the methods for all meat types. This may also help understand why the cooked meat extraction method worked better for PLSR prediction of beef mixtures compared to the poultry mixtures.

At the lowest adulterant concentration of 0.5% w/w and irrespective of the protein base, benchtop spectrophotometer could classify all the different adulterant mixtures with accuracies of 99.47% average recognition and 98.75% average prediction. Classification results using the handheld spectrophotometer yielded accuracies of 65.13% average recognition and 53.49% average prediction for the optical glass cuvette and average recognition 83.79% and average prediction 56.19% for the LDPE plastic bag. Benchtop spectrophotometer gave the best LDA accuracies for classifying single, dual, triple and quadruple mixtures of urea, glycine, taurine and melamine and that lowest concentration. of 0.5% w/w. Using the handheld spectrophotometer with LPDE plastic bag gave better accuracies than using the handheld spectrophotometer with optical glass. Using the benchtop spectrophotometer gave the best model for predicting protein powder concentrations in all the samples with R^2CV 0.86, RMSECV 1.36% w/w, R^2pred 0.87 and RMSEP 1.30% w/w. Using the handheld spectrophotometer with optical glass gave R^2CV 0.84, RMSECV

1.38% w/w, R^2pred 0.84 and RMSEP 1.38% w/w for predicting protein powder concentrations in all the samples. Using the handheld spectrophotometer with optical LDPE plastic gave R^2CV 0.86, RMSECV 1.39% w/w, R^2pred 0.84 and RMSEP 1.47% w/w for predicting protein powder concentrations in all the samples. Based on the R^2CV and RMSECV values using the LDPE plastic bag gave the results for predicting protein powder in the samples compared to using the handheld spectrophotometer with optical glass. They both however, had similar R^2pred and RMSEP values. Using the benchtop spectrophotometer gave the best model for predicting protein powder concentrations in protein powder mixtures that contained only single adulterants of urea, glycine, taurine and melamine at concentrations of 0.5-3% w/w. There was R^2CV 0.90, RMSECV 1.41% w/w, R^2pred 0.93 and RMSEP 1.22% w/w. Using the handheld spectrophotometer with optical glass gave R^2CV 0.84, RMSECV 1.18% w/w, R^2pred 0.88 and RMSEP 1.58% w/w for predicting protein powder concentrations in protein powder mixtures that contained single mixtures of urea, glycine, taurine and melamine at concentrations of 0.5–3% w/w. Using the handheld spectrophotometer with optical LDPE plastic gave R^2CV 0.91, RMSECV 1.36% w/w, R^2pred 0.92 and RMSEP 1.33% w/w for predicting protein powder concentrations in protein powder mixtures that contained single mixtures of urea, glycine, taurine and melamine at concentrations of 0.5-3% w/w. Based on the R^2CV, RMSECV , R^2pred and RMSEP values, using the LDPE plastic bag gave the results compared to using the handheld spectrophotometer with optical glass for predicting protein powder concentrations in protein powder mixtures that contained single mixtures of urea, glycine, taurine and melamine at concentrations of 0.5-3% w/w.

Benchtop spectrophotometer yielded the lowest limit of detections (LOD's) and limit of quantifications (LOQ's) for quantifying urea, glycine, taurine and melamine in whey, beef and beef protein powder compared to when the handheld spectrophotometer was used. Comparatively, using the handheld spectrophotometer and optical glass yielded lower LODmin/max and LOQmin/max for some of the adulterants than when the handheld spectrophotometer was used with optical glass was used but samples scanned through the LDPE plastic bag, had a better repeatability when average LOD and LOQ's were evaluated. Urea and melamine had the lowest LOD and LOQ irrespective of the instrument or scanning method. Taurine always had the highest LOD and LOQ irrespective of the instrument or scanning method. For future studies regarding protein powder adulteration, it is recommended to examine practically worthy levels of protein powder adulteration so that specific models may be developed for them with well-defined target accuracies.

9. LIST OF PUBLICATIONS IN THE FIELD OF STUDIES

1. **John Lewis Zinia Zaukuu**, Zoltan Gillay, Zoltan Kovacs. Standardized Extraction Techniques for Meat Analysis with the Electronic Tongue: A Case Study of Poultry and Red Meat Adulteration. Volume 21, issue 2, page 481. https://doi.org/10.3390/s21020481. **Sensors (2021). Q1, IF: 3.240**
2. **John-Lewis Zinia Zaukuu,** Balkis Aouadi, Mátyás Lukács, Zsanett Bodor, Flóra Vitális, Biborka Gillay, Zoltan Gillay, László Friedrich, Zoltan Kovacs. Detecting Low Concentrations of Nitrogen-Based Adulterants in Whey Protein Powder Using Benchtop and Handheld NIR Spectrophotometers and the Feasibility of Scanning through Plastic Bag. Volume 25, issue 11, pages 2522. https://doi.org/10.3390/molecules25112522 . **Molecules (2020). Q2, IF: 3.267**
3. Balkis Aouadi, **John-Lewis Zinia Zaukuu**, Flora Vitális, Zsanett Bodor, Orsolya Fehér, Zoltan Gillay, George Bazar, Zoltan Kovacs. Historical Evolution and Food Control Achievements of Near Infrared Spectroscopy, Electronic Nose, and Electronic Tongue—Critical Overview. Volume 20, issue 17, Pages 5479. https://doi.org/10.3390/s20195479 **Sensors (2020). Q1, IF: 3.240**
4. Zoltan Kovacs, Dániel Szöllősi, **John-Lewis Zinia Zaukuu,** Zsanett Bodor, Flóra Vitális, Balkis Aouadi, Viktória Zsom-Muha, Zoltan Gillay. Factors influencing the long-term stability of electronic tongue and application of improved drift correction methods. Biosensors (2020). Volume 10, issue 7, pages 74. https://doi.org/10.3390/bios10070074 **Biosensors (2020). Q2, IF: 3.275**
5. **John-Lewis Zinia Zaukuu**, János Soós, Zsanett Bodor, József Felföldi, Ildikó Magyar, Zoltan Kovacs. Authentication of Tokaji Wine (Hungaricum) with the Electronic Tongue and Near Infrared Spectroscopy. Volume 84, issue 12, page 3437-3444. https://doi.org/10.1111/1750-3841.14956. **Journal of Food Science (2019). Q2, IF: 2.478**
6. **John Lewis Zinia Zaukuu**, George Bazar, Zoltan Gillay, Zoltan Kovacs. Emerging trends of advanced sensor-based instruments for meat, poultry and fish quality–a review. Volume 60, issue 12, Pages 3443-3460. https://doi.org/10.1080/10408398.2019.1691972. **Critical Reviews in Food Science and Nutrition (2019). Q1, IF: 7.862**

10. APPENDICES

10.1. A1: Bibliography

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10.2. A2

Table 30: Confusion matrix using the benchtop spectrophotometer to classify authentic Tokaji wine and Forditas I wine that was adulterated with grape must concentrate and sucrose to mimic the sugar concentration of the authentic wines. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%)

Accuracies		Aszu_I	Aszu_II	Forditas_I	Forditas_II	Base_Sugar	Forditas_I C1	Forditas_I C2	Forditas_I C3	Forditas_I C4
Average recognition 97.01%	Aszu_I	100	0	0	0	0	11.17	0	0	0
	Aszu_II	0	100	0	0	0	0	0	0	0
	Forditas_I	0	0	100	0	0	0	0	0	0
	Forditas_II	0	0	0	100	0	0	0	0	0
	Base_Sugar	0	0	0	0	100	0	0	0	15.8
	Forditas_I_C1	0	0	0	0	0	88.83	0	0	0
	Forditas_I_C2	0	0	0	0	0	0	100	0	0
	Forditas_I_C3	0	0	0	0	0	0	0	100	0
	Forditas_I_C4	0	0	0	0	0	0	0	0	84.2
Average prediction 96.18%	Aszu_I	100	0	0	0	0	0	0	0	0
	Aszu_II	0	100	0	0	0	0	0	0	0
	Forditas_I	0	0	100	0	0	0	0	0	0
	Forditas_II	0	0	0	100	0	0	0	0	0
	Base_Sugar	0	0	0	0	100	0	0	11	12.41
	Forditas_I_C1	0	0	0	0	0	89	0	0	0
	Forditas_I_C2	0	0	0	0	0	11	100	0	0
	Forditas_I_C3	0	0	0	0	0	0	0	89	0
	Forditas_I_C4	0	0	0	0	0	0	0	0	87.59

Table 31: Confusion matrix using the handheld spectrophotometer to classify authentic Tokaji wine and Forditas I wine that was adulterated with grape must concentrate and sucrose to mimic the sugar concentration of the authentic wines. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%)

Accuracies		Aszu_I	Aszu_II	Forditas_I	Forditas_II	Base_Sugar	Forditas_I C1	Forditas_I C2	Forditas_I C3	Forditas_I C4
Average recognition 72.40%	Aszu_I	59.97	0	0	5.5	33.33	5.51	16.67	0	0
	Aszu_II	0	83.33	0	0	11.17	0	0	8.25	30.72
	Forditas_I	0	0	100	16.67	0	0	0	0	0
	Forditas_II	10.04	0	0	66.67	0	0	0	0	0
	Base_Sugar	0	5.5	0	0	55.5	0	0	0	0
	Forditas_I_C1	0	0	0	0	0	66.78	0	0	0
	Forditas_I_C2	19.94	0	0	11.17	0	22.2	83.33	25	0
	Forditas_I_C3	10.04	11.17	0	0	0	5.51	0	66.75	0
	Forditas_I_C4	0	0	0	0	0	0	0	0	69.28
Average prediction 68.22%	Aszu_I	70.18	0	11	0	33.44	11.04	11	0	0
	Aszu_II	0	77.93	0	0	11.04	0	0	16.5	40.12
	Forditas_I	0	0	89	22.33	0	0	0	0	0
	Forditas_II	9.94	0	0	66.67	0	0	0	0	0
	Base_Sugar	0	11.04	0	0	44.48	0	0	0	0
	Forditas_I_C1	0	0	0	0	11.04	66.89	0	0	0
	Forditas_I_C2	9.94	0	0	11	0	11.04	89	33.5	0
	Forditas_I_C3	9.94	11.04	0	0	0	11.04	0	50	0
	Forditas_I_C4	0	0	0	0	0	0	0	0	51.88

10.3. A3

Table 32: Confusion matrix using the benchtop spectrophotometer to classify authentic Tokaji wine and Forditas II wine that was adulterated with grape must concentrate and sucrose to mimic the sugar concentration of the authentic wines. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%)

Accuracies		Aszu_I	Aszu_II	Forditas_I	Forditas_II	Base_Sugar	Forditas_II C1	Forditas_II C2	Forditas_II C3	Forditas_II C4
Average recognition 98.76%	Aszu_I	100	0	0	0	0	0	0	0	0
	Aszu_II	0	100	0	0	0	0	0	0	0
	Forditas_I	0	0	100	0	0	0	0	0	0
	Forditas_II	0	0	0	100	0	0	0	0	0
	Base_Sugar	0	0	0	0	100	0	11.17	0	0
	Forditas_II_C1	0	0	0	0	0	100	0	0	0
	Forditas_II_C2	0	0	0	0	0	0	88.83	0	0
	Forditas_II_C3	0	0	0	0	0	0	0	100	0
	Forditas_II_C4	0	0	0	0	0	0	0	0	100
Average prediction 98.78%	Aszu_I	100	0	0	0	0	0	0	0	0
	Aszu_II	0	100	0	0	0	0	0	0	0
	Forditas_I	0	0	100	0	0	0	0	0	0
	Forditas_II	0	0	0	100	0	0	0	0	0
	Base_Sugar	0	0	0	0	100	0	11	0	0
	Forditas_II_C1	0	0	0	0	0	100	0	0	0
	Forditas_II_C2	0	0	0	0	0	0	89	0	0
	Forditas_II_C3	0	0	0	0	0	0	0	100	0
	Forditas_II_C4	0	0	0	0	0	0	0	0	100

Table 33: Confusion matrix using the handheld spectrophotometer to classify authentic Tokaji wine and Forditas II wine that was adulterated with grape must concentrate and sucrose to mimic the sugar concentration of the authentic wines. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%)

Accuracies		Aszu_I	Aszu_II	Forditas_I	Forditas_II	Base_Sugar	Forditas_II C1	Forditas_II C2	Forditas_II C3	Forditas_II C4
Average recognition 81.87%	Aszu_I	89.96	0	0	0	11.15	27.83	0	0	0
	Aszu_II	0	83.33	0	0	11.15	0	34.98	12.55	0
	Forditas_I	0	0	100	33.33	0	0	0	0	0
	Forditas_II	0	0	0	66.67	0	0	0	0	0
	Base_Sugar	0	11.17	0	0	77.7	5.5	0	0	0
	Forditas_II_C1	0	0	0	0	0	66.67	0	0	0
	Forditas_II_C2	0	0	0	0	0	0	65.02	0	0
	Forditas_II_C3	10.04	5.5	0	0	0	0	0	87.45	0
	Forditas_II_C4	0	0	0	0	0	0	0	0	100
Average prediction 76.07%	Aszu_I	80.18	0	0	11.04	11	22.33	0	0	0
	Aszu_II	0	89	0	0	0	0	29.94	12.41	0
	Forditas_I	0	0	100	33.44	0	0	0	0	0
	Forditas_II	9.91	0	0	44.48	0	0	0	0	0
	Base_Sugar	0	11	0	11.04	66.67	11	20.06	0	0
	Forditas_II_C1	0	0	0	0	0	66.67	0	0	0
	Forditas_II_C2	0	0	0	0	22.33	0	50	0	0
	Forditas_II_C3	9.91	0	0	0	0	0	0	87.59	0
	Forditas_II_C4	0	0	0	0	0	0	0	0	100

10.4. A4

Table 34: Confusion matrix using the electronic tongue to classify authentic Tokaji wine and Forditas I wine that was adulterated with grape must concentrate and sucrose to mimic the sugar concentration of the authentic wines. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%)

Accuracies		Aszu_I	Aszu_II	Forditas_I	Forditas_II	Base_Sugar	Forditas_I C1	Forditas_I C2	Forditas_I C3	Forditas_I C4
Average recognition 99.54%	Aszu_I	100	0	0	0	0	0	0	0	0
	Aszu_II	0	100	0	0	0	0	0	0	0
	Forditas_I	0	0	100	0	0	0	0	0	0
	Forditas_II	0	0	0	100	0	0	0	0	0
	Base_Sugar	0	0	0	0	100	0	0	0	0
	Forditas_I_C1	0	0	0	0	0	100	0	0	0
	Forditas_I_C2	0	0	0	0	0	0	100	0	0
	Forditas_I_C3	0	0	0	0	0	0	0	100	4.12
	Forditas_I_C4	0	0	0	0	0	0	0	0	95.88
Average prediction 98.17%	Aszu_I	100	0	0	0	0	0	0	0	0
	Aszu_II	0	100	0	0	0	0	0	0	0
	Forditas_I	0	0	100	0	0	0	0	0	0
	Forditas_II	0	0	0	100	0	0	0	0	0
	Base_Sugar	0	0	0	0	100	0	0	0	0
	Forditas_I_C1	0	0	0	0	0	100	0	0	0
	Forditas_I_C2	0	0	0	0	0	0	100	0	0
	Forditas_I_C3	0	0	0	0	0	0	0	91.75	8.25
	Forditas_I_C4	0	0	0	0	0	0	0	8.25	91.75

Table 35: Confusion matrix using the electronic tongue to classify authentic Tokaji wine and Forditas II wine that was adulterated with grape must concentrate and sucrose to mimic the sugar concentration of the authentic wines. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%)

Accuracies		Aszu_I	Aszu_II	Forditas_I	Forditas_II	Base_Sugar	Forditas_II C1	Forditas_II C2	Forditas_II C3	Forditas_II C4
Average recognition 100%	Aszu_I	100	0	0	0	0	0	0	0	0
	Aszu_II	0	100	0	0	0	0	0	0	0
	Forditas_I	0	0	100	0	0	0	0	0	0
	Forditas_II	0	0	0	100	0	0	0	0	0
	Base_Sugar	0	0	0	0	100	0	0	0	0
	Forditas_II_C1	0	0	0	0	0	100	0	0	0
	Forditas_II_C2	0	0	0	0	0	0	100	0	0
	Forditas_II_C3	0	0	0	0	0	0	0	100	0
	Forditas_II_C4	0	0	0	0	0	0	0	0	100
Average prediction 93.17%	Aszu_I	100	0	0	0	0	0	0	0	0
	Aszu_II	0	100	0	0	0	0	0	0	0
	Forditas_I	0	0	100	0	0	0	0	0	0
	Forditas_II	0	0	0	46.19	7.62	0	0	0	0
	Base_Sugar	0	0	0	53.81	92.38	0	0	0	0
	Forditas_II_C1	0	0	0	0	0	100	0	0	0
	Forditas_II_C2	0	0	0	0	0	0	100	0	0
	Forditas_II_C3	0	0	0	0	0	0	0	100	0
	Forditas_II_C4	0	0	0	0	0	0	0	0	100

10.5. A5

Table 36: Electronic tongue signal correction using the additive correction relative to all samples (Kovacs et al., 2020) for datasets from the Tokaji wine experiment

	With no drift correction		With drift correction	
	Average recognition (%)	Average Prediction (%)	Average recognition (%)	Average Prediction (%)
Forditas I wine adulteration	98.66	94.32	99.54	98.16
Forditas II wine adulteration	99.54	89.69	100	93.17

10.6. A6

Table 37: Electronic tongue signal correction using the additive correction relative to all samples (Kovacs et al., 2020) for datasets from the meat experiment for determination of optimal extraction method

		With drift correction		With no drift correction	
		Average recognition (%)	Average Prediction (%)	Average recognition (%)	Average Prediction (%)
Chicken and turkey mixtures	Raw meat extraction with distilled water	81.28	58.34	91.03	33.37
	Frozen meat extraction with distilled water	88.58	62.55	72.92	31.25
	Meat extraction by cooking with distilled water	78.13	64.72	77.07	39.61
Pork and beef mixtures	Raw meat extraction with distilled water	67.73	54.25	53.16	18.81
	Frozen meat extraction with distilled water	85.41	56.31	72.96	25.02
	Meat extraction by cooking with distilled water	89.62	66.77	65.70	24.94

10.7. A7

Table 38: Confusion matrix (calibration results) using the benchtop spectrophotometer and optical glass to classify authentic protein powders and 16 different adulterated protein powder mixture containing urea, glycine, taurine and melamine at their lowest concentrations of 0.5%w/w. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%). Average calibration: 99.47%

	G	GM	GT	GTM	M	Pure beef	Pure pea	Pure whey	T	TM	U	UG	UGM	UGT	UGTM	UM	UT	UTM
G	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GM	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GT	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GTM	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0
Pure beef	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Pure pea	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0
Pure whey	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0
T	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0
TM	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0
U	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0
UG	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
UGM	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0
UGT	0	0	0	0	0	0	0	0	0	0	0	0	0	96.14	0	0	0	0
UGTM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	98.17	0	3.72	0
UM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0
UT	0	0	0	0	0	0	0	0	0	0	0	0	0	3.86	1.83	0	96.28	0
UTM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100

Table 39: Confusion matrix (prediction results) using the benchtop spectrophotometer and optical glass to classify authentic protein powders and 16 different adulterated protein powder mixture containing urea, glycine, taurine and melamine at their lowest concentrations of 0.5%w/w. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%). Average prediction: 98.75%

	G	GM	GT	GTM	M	Pure beef	Pure pea	Pure whey	T	TM	U	UG	UGM	UGT	UGTM	UM	UT	UTM
G	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GM	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GT	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GTM	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
M	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0
Pure beef	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0
Pure pea	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0
Pure whey	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0
T	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0
TM	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0
U	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	3.67	0	0
UG	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0
UGM	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0
UGT	0	0	0	0	0	0	0	0	0	0	0	0	0	96.19	0	0	0	0
UGTM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	7.44	0
UM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	96.33	0	0
UT	0	0	0	0	0	0	0	0	0	0	0	0	0	3.81	0	0	92.56	7.44
UTM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	92.56

Table 40: Confusion matrix (calibration results) using the handheld spectrophotometer and optical glass to classify authentic protein powders and 16 different adulterated protein powder mixture containing urea, glycine, taurine and melamine at their lowest concentrations of 0.5%w/w. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%). Average calibration: 65.13%

	G	GM	GT	GTM	M	Pure beef	Pure pea	Pure whey	T	TM	U	UG	UGM	UGT	UGTM	UM	UT	UTM
G	81.45	0	14.83	0	0	0	5.51	0	1.83	0	0	0	0	0	3.72	0	0	0
GM	1.83	62.98	0	5.56	14.83	0	5.51	5.5	1.83	1.83	0	3.72	7.39	5.56	1.83	1.83	0	9.28
GT	3.72	0	64.8	0	0	0	0	0	20.4	1.83	0	0	0	0	1.83	0	0	0
GTM	0	0	3.72	55.59	0	0	0	0	3.72	7.39	0	0	1.83	7.39	1.83	0	0	3.72
M	0	11.12	0	0	79.61	0	0	0	0	7.39	0	0	7.39	0	0	12.95	0	1.83
Pure beef	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	1.83	0	0
Pure pea	0	0	0	0	0	0	88.98	0	0	3.72	0	0	1.83	0	0	0	0	0
Pure whey	9.27	1.83	5.55	1.83	0	0	0	94.5	1.83	0	0	0	0	0	0	0	0	1.83
T	0	0	9.27	9.28	0	0	0	0	66.7	1.83	0	0	0	0	0	0	1.9	1.83
TM	3.72	1.83	1.83	7.39	0	0	0	0	0	68.54	0	0	0	9.28	0	0	11.53	0
U	0	0	0	0	0	0	0	0	0	0	63.67	1.83	3.72	1.83	0	20.4	5.76	0
UG	0	1.83	0	1.83	0	0	0	0	0	0	5.46	70.39	22.23	5.56	3.72	3.72	5.76	0
UGM	0	0	0	0	1.83	0	0	0	0	0	5.46	7.39	29.63	0	3.72	1.83	9.63	3.72
UGT	0	14.84	0	7.39	0	0	0	0	0	0	0	5.56	14.84	51.86	7.39	0	15.39	0
UGTM	0	1.83	0	5.56	0	0	0	0	1.83	3.72	0	5.56	5.56	9.28	62.94	3.72	9.63	7.39
UM	0	0	0	0	0	0	0	0	0	0	18.17	1.83	3.72	1.83	3.72	44.47	3.86	1.83
UT	0	3.72	0	1.83	0	0	0	0	1.83	0	0	0	0	5.56	3.72	1.83	26.92	9.28
UTM	0	0	0	3.72	3.72	0	0	0	0	3.72	7.26	3.72	1.83	1.83	5.56	7.39	9.63	59.28

Table 41: Confusion matrix (prediction results) using the handheld spectrophotometer and optical glass to classify authentic protein powders and 15 different adulterated protein powder mixture containing urea, glycine, taurine and melamine at their lowest concentrations of 0.5%w/w. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%). Average prediction: 53.49%

	G	GM	GT	GTM	M	Pure beef	Pure pea	Pure whey	T	TM	U	UG	UGM	UGT	UGTM	UM	UT	UTM
G	74.11	0	18.56	0	0	0	11	11.04	0	0	0	3.67	3.67	0	3.67	0	0	0
GM	0	55.56	0	14.78	14.81	0	0	11.04	3.67	18.6	0	7.45	11.14	18.56	3.67	0	0	7.44
GT	7.44	0	51.89	3.67	0	0	0	0	18.58	3.67	0	0	0	0	0	0	0	0
GTM	0	3.67	3.67	29.67	0	0	0	0	3.67	3.67	0	0	0	7.44	3.67	0	0	11.1
M	0	11.11	0	0	66.82	0	0	0	0	14.81	0	0	7.46	0	0	14.79	0	7.44
Pure beef	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	3.67	0	0
Pure pea	0	0	0	0	0	0	89	0	0	0	0	0	3.67	0	0	0	0	0
Pure whey	14.78	3.67	3.67	0	0	0	0	66.89	0	0	0	0	3.67	0	0	0	0	0
T	0	0	14.78	14.78	0	0	0	0	66.74	3.67	0	0	0	0	0	0	3.81	0
TM	3.67	7.44	7.44	11.11	3.67	0	0	0	3.67	48.22	0	0	3.67	7.44	0	0	11.55	0
U	0	0	0	0	3.67	0	0	0	0	0	61.48	3.67	7.46	0	3.67	25.92	7.74	0
UG	0	7.44	0	0	0	0	0	0	0	0	7.73	70.41	14.81	3.67	3.67	3.67	11.55	0
UGM	0	0	0	0	0	0	0	11.04	0	0	7.73	0	11.14	0	3.67	11.12	3.81	7.44
UGT	0	0	0	7.44	0	0	0	0	3.67	0	0	0	14.81	37	11.14	0	23.09	11.1
UGTM	0	7.44	0	7.44	3.67	0	0	0	0	3.67	0	3.67	11.14	11.11	52	3.67	15.36	3.66
UM	0	0	0	0	3.67	0	0	0	0	0	15.34	0	3.67	3.67	0	29.7	0	7.44
UT	0	3.67	0	3.67	3.67	0	0	0	0	0	0	0	3.67	7.44	3.67	0	15.36	7.44
UTM	0	0	0	7.44	0	0	0	0	0	3.67	7.73	11.12	0	3.67	11.14	7.45	7.74	36.96

Table 42: Confusion matrix (calibration results) using the handheld spectrophotometer and LDPE plastic to classify authentic protein powders and 15 different adulterated protein powder mixture containing urea, glycine, taurine and melamine at their lowest concentrations of 0.5%w/w. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%). Average calibration: 83.79%

	G	GM	GT	GTM	M	Pure beef	Pure pea	Pure whey	T	TM	U	UG	UGM	UGT	UGTM	UM	UT	UTM
G	83.36	0	7.39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GM	2.06	81.55	0	11.11	3.72	0	0	0	0	1.84	0	0	5.56	2.36	1.65	0	1.83	1.83
GT	0	3.72	75.99	3.72	0	0	0	0	9.11	5.56	0	0	0	2.36	5	0	0	0
GTM	8.32	1.83	1.83	74.06	0	0	0	0	3.65	5.56	0	0	0	0	0	0	0	0
M	0	0	0	0	94.44	0	0	0	0	3.73	0	0	1.83	0	3.35	0	0	0
Pure beef	2.06	0	0	0	0	100	0	0	0	1.84	0	0	0	0	0	0	0	0
Pure pea	0	1.83	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0
Pure whey	4.19	1.83	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0
T	0	0	7.39	0	0	0	0	0	87.24	0	0	0	0	0	0	0	3.72	1.83
TM	0	0	5.56	11.11	0	0	0	0	0	57.45	0	0	3.72	0	5	0	0	3.72
U	0	0	0	0	0	0	0	0	0	0	100	1.83	0	0	1.65	2.06	0	0
UG	0	1.83	0	0	0	0	0	0	0	0	0	81.55	7.39	4.79	1.65	6.25	5.56	0
UGM	0	0	0	0	1.83	0	0	0	0	0	0	9.28	62.94	4.79	1.65	0	3.72	3.72
UGT	0	0	0	0	0	0	0	0	0	1.84	0	1.83	0	73.79	0	0	5.56	5.56
UGTM	0	1.83	0	0	0	0	0	0	0	1.84	0	0	7.39	2.36	63.38	0	1.83	9.28
UM	0	0	0	0	0	0	0	0	0	0	0	1.83	3.72	0	6.65	91.69	0	1.83
UT	0	0	1.83	0	0	0	0	0	0	1.84	0	1.83	3.72	4.79	5	0	68.5	0
UTM	0	5.56	0	0	0	0	0	0	0	18.52	0	1.83	3.72	4.79	5	0	9.28	72.22

Table 43: Confusion matrix (prediction results) using the handheld spectrophotometer and LDPE plastic to classify authentic protein powders and 16 different adulterated protein powder mixture containing urea, glycine, taurine and melamine at their lowest concentrations of 0.5%w/w. Columns represent the actual class membership (%) and the rows represent the predicted class membership (%). Average prediction: 56.19%

	G	GM	GT	GTM	M	Pure beef	Pure pea	Pure whey	T	TM	U	UG	UGM	UGT	UGTM	UM	UT	UTM
G	70.88	11.12	7.45	3.67	0	0	0	11.04	0	0	0	0	0	0	0	0	0	0
GM	8.38	48.16	3.67	18.58	14.78	0	11.04	0	0	0	0	3.67	7.44	4.72	6.69	0	0	0
GT	0	0	55.62	0	0	0	0	0	19.26	7.45	0	0	0	4.72	9.99	0	0	0
GTM	4.12	7.45	3.67	37.04	0	0	0	0	7.73	11.12	0	0	0	0	0	0	7.44	11.12
M	0	14.79	0	0	66.67	0	11.04	0	0	3.67	0	0	0	0	0	12.5	0	3.67
Pure beef	4.12	0	0	7.45	0	89	0	11.04	0	3.67	0	0	3.67	0	0	0	0	3.67
Pure pea	0	0	0	3.67	7.44	0	66.89	11.04	3.81	0	0	0	0	0	0	0	0	0
Pure whey	12.5	3.67	0	0	0	0	11.04	66.89	0	0	0	0	0	0	0	0	0	0
T	0	0	14.79	3.67	0	0	0	0	65.4	3.67	0	0	0	0	0	0	3.66	3.67
TM	0	0	11.12	7.45	0	0	0	0	0	37.04	0	0	3.67	4.72	0	0	0	0
U	0	0	0	0	0	0	0	0	0	0	95.88	3.67	0	0	3.3	16.62	3.66	0
UG	0	0	0	0	0	0	0	0	0	0	4.12	55.56	7.44	9.59	6.69	8.38	18.53	7.45
UGM	0	0	0	0	0	0	0	0	0	0	0	3.67	37	4.72	6.69	0	7.44	7.45
UGT	0	3.67	3.67	11.12	0	0	0	0	3.81	3.67	0	7.44	3.67	33.33	0	0	7.44	0
UGTM	0	7.45	0	3.67	0	0	0	0	0	7.45	0	7.44	18.56	4.72	29.97	0	0	3.67
UM	0	0	0	0	0	0	0	0	0	0	0	11.11	7.44	0	9.99	58.38	0	3.67
UT	0	0	0	3.67	0	0	0	0	0	7.45	0	7.44	0	23.89	9.99	0	40.73	18.58
UTM	0	3.67	0	0	11.11	11	0	0	0	14.79	0	0	11.11	9.59	16.68	4.12	11.1	37.04

Table 44: PLSR models for adulterated whey protein powder samples scanned with the benchtop spectrophotometer and analyzed at the spectral range of 950-1650 nm

Models	Adulterant	LV	R^2	RMSEC (% w/w)	R^2CV	RMSECV (% w/w)
Whole data	Urea	5	0.93	0.22	0.88	0.29
	Glycine	7	0.91	0.63	0.84	0.86
	Taurine	10	0.93	0.86	0.84	1.40
	Melamine	5	0.92	0.17	0.87	0.21
Only Single mixtures	Urea	6	0.99	0.12	0.61	0.76
	Glycine	3	0.88	1.05	0.46	2.23
	Taurine	6	0.98	0.75	0.51	3.55
	Melamine	3	0.87	0.31	0.65	0.50
Only Dual mixtures	Urea	3	0.90	0.19	0.80	0.27
	Glycine	2	0.88	0.52	0.82	0.65
	Taurine	2	0.87	0.90	0.82	1.08
	Melamine	4	0.89	0.14	0.70	0.24
Only Multiple mixtures	Urea	3	0.85	0.15	0.79	0.17
	Glycine	7	0.89	0.32	0.79	0.45
	Taurine	3	0.83	0.67	0.78	0.76
	Melamine	10	0.95	0.06	0.77	0.13

Table 45: PLSR models for adulterated whey protein powder samples scanned with the handheld spectrophotometer through the optical glass and analyzed at the spectral range of 950-1650 nm

Model dataset	Predicted adulterant	LV	R^2	RMSEC (% w/w)	R^2CV	RMSECV (% w/w)
Whole data	Urea	17	0.94	0.19	0.89	0.27
	Glycine	13	0.84	0.83	0.79	0.93
	Taurine	11	0.86	1.31	0.81	1.52
	Melamine	14	0.89	0.19	0.82	0.25
Only Single mixtures	Urea	7	0.92	0.34	0.84	0.49
	Glycine	7	0.82	1.32	0.74	1.59
	Taurine	6	0.80	2.29	0.61	3.24
	Melamine	6	0.86	0.32	0.59	0.55
Only Dual mixtures	Urea	7	0.88	0.26	0.85	0.29
	Glycine	10	0.81	0.73	0.68	0.95
	Taurine	7	0.81	1.39	0.71	1.69
	Melamine	7	0.83	0.22	0.77	0.25
Only Multiple mixtures	Urea	10	0.88	0.16	0.79	0.21
	Glycine	12	0.83	0.49	0.69	0.65
	Taurine	10	0.82	0.83	0.67	1.12
	Melamine	11	0.81	0.14	0.68	0.19

Table 46: PLSR models for adulterated whey protein powder samples scanned with the handheld spectrophotometer through the LDPE plastic bag and analyzed at the spectral range of 950-1650 nm.

Model dataset	Predicted adulterant	LV	R^2	RMSEC (% w/w)	R^2CV	RMSECV (% w/w)
Whole data	Urea	11	0.95	0.19	0.92	0.23
	Glycine	17	0.86	0.79	0.74	1.09
	Taurine	13	0.85	1.37	0.78	1.66
	Melamine	8	0.82	0.26	0.78	0.28
Only Single mixtures	Urea	5	0.96	0.24	0.92	0.35
	Glycine	5	0.79	1.40	0.67	1.79
	Taurine	4	0.87	1.87	0.60	3.27
	Melamine	5	0.92	0.25	0.84	0.35
Only Dual mixtures	Urea	7	0.95	0.18	0.91	0.23
	Glycine	7	0.85	0.75	0.78	0.89
	Taurine	8	0.88	1.12	0.80	1.42
	Melamine	7	0.87	0.19	0.83	0.22
Only Multiple mixtures	Urea	6	0.90	0.15	0.83	0.19
	Glycine	10	0.74	0.62	0.49	0.86
	Taurine	8	0.74	0.98	0.57	1.27
	Melamine	8	0.73	0.17	0.54	0.23

Table 47: PLSR models for adulterated beef protein powder samples scanned with the benchtop spectrophotometer and analyzed at the spectral range of 950-1650 nm

Models	Adulterant	LV	R^2	RMSEC (% w/w)	R^2CV	RMSECV (% w/w)
Whole data	Urea	8	0.95	0.19	0.85	0.33
	Glycine	4	0.90	0.68	0.83	0.90
	Taurine	12	0.98	0.53	0.87	1.25
	Melamine	5	0.75	0.29	0.52	0.41
Only Single mixtures	Urea	1	0.87	0.44	0.26	1.05
	Glycine	6	0.99	0.16	0.22	2.69
	Taurine	6	0.99	0.24	0.69	2.84
	Melamine	2	0.95	0.19	0.74	0.44
Only Dual mixtures	Urea	3	0.92	0.17	0.84	0.24
	Glycine	6	0.95	0.34	0.54	1.04
	Taurine	3	0.87	0.90	0.69	1.40
	Melamine	3	0.88	0.15	0.78	0.19
Only Multiple mixtures	Urea	3	0.87	0.14	0.81	0.17
	Glycine	12	0.99	0.10	0.79	0.45
	Taurine	4	0.87	0.59	0.79	0.74
	Melamine	3	0.82	0.11	0.74	0.14

Table 48: PLSR models for adulterated beef protein powder samples scanned with the handheld spectrophotometer through the optical glass and analyzed at the spectral range of 950-1650 nm.

Model dataset	Predicted adulterant	LV	R^2	RMSEC (% w/w)	R^2CV	RMSECV (% w/w)
Whole data	Urea	15	0.92	0.24	0.89	0.27
	Glycine	9	0.83	0.84	0.79	0.94
	Taurine	13	0.88	1.24	0.83	1.44
	Melamine	13	0.78	0.28	0.70	0.32
Only Single mixtures	Urea	7	0.92	0.35	0.76	0.61
	Glycine	6	0.91	0.92	0.85	1.22
	Taurine	7	0.90	1.63	0.74	2.65
	Melamine	7	0.85	0.33	0.68	0.49
Only Dual mixtures	Urea	7	0.86	0.29	0.79	0.34
	Glycine	9	0.76	0.83	0.62	1.04
	Taurine	7	0.83	1.32	0.74	1.61
	Melamine	7	0.66	0.31	0.34	0.43
Only Multiple mixtures	Urea	13	0.86	0.18	0.75	0.24
	Glycine	9	0.81	0.51	0.75	0.59
	Taurine	9	0.81	0.85	0.69	1.08
	Melamine	12	0.78	0.15	0.61	0.20

Table 49: PLSR models for adulterated beef protein powder samples scanned with the handheld spectrophotometer through the LDPE plastic bag and analyzed at the spectral range of 950-1650 nm

Model dataset	Predicted adulterant	LV	R^2	RMSEC (% w/w)	R^2CV	RMSECV (% w/w)
Whole data	Urea	17	0.94	0.19	0.93	0.23
	Glycine	16	0.79	0.97	0.73	1.11
	Taurine	16	0.82	1.48	0.75	1.74
	Melamine	13	0.74	0.31	0.66	0.35
Only Single mixtures	Urea	7	0.92	0.37	0.79	0.57
	Glycine	5	0.82	1.34	0.72	1.68
	Taurine	7	0.88	1.77	0.64	3.09
	Melamine	7	0.84	0.35	0.74	0.45
Only Dual mixtures	Urea	7	0.84	0.31	0.79	0.35
	Glycine	7	0.71	1.03	0.58	1.23
	Taurine	8	0.83	1.33	0.75	1.61
	Melamine	7	0.60	0.34	0.23	0.47
Only Multiple mixtures	Urea	11	0.89	0.15	0.82	0.19
	Glycine	12	0.62	0.73	0.39	0.93
	Taurine	19	0.83	0.78	0.61	1.19
	Melamine	12	0.71	0.18	0.51	0.23

Table 50: PLSR models for adulterated pea protein powder samples scanned with the benchtop spectrophotometer and analyzed at the spectral range of 950-1650 nm

Models	Adulterant	LV	R^2	RMSEC (% w/w)	R^2CV	RMSECV (% w/w)
(1) Whole data	Urea	5	0.93	0.22	0.88	0.29
	Glycine	3	0.92	0.64	0.85	0.89
	Taurine	6	0.93	0.97	0.85	1.34
	Melamine	5	0.92	0.16	0.89	0.19
Only Single mixtures	Urea	1	0.89	0.39	0.60	0.76
	Glycine	4	0.97	0.55	0.48	2.28
	Taurine	1	0.88	1.73	0.41	3.90
	Melamine	1	0.88	0.29	0.71	0.46
Only Dual mixtures	Urea	3	0.91	0.18	0.84	0.25
	Glycine	4	0.94	0.38	0.80	0.68
	Taurine	3	0.88	0.86	0.77	1.21
	Melamine	3	0.91	0.13	0.83	0.17
Only Multiple mixtures	Urea	3	0.85	0.15	0.79	0.18
	Glycine	4	0.88	0.33	0.82	0.42
	Taurine	2	0.85	0.64	0.79	0.74
	Melamine	4	0.83	0.11	0.75	0.14

Table 51: PLSR models for adulterated pea protein powder samples scanned with the handheld spectrophotometer through the optical glass and analyzed at the spectral range of 950-1650 nm

Model dataset	Predicted adulterant	LV	R^2	RMSEC (% w/w)	R^2CV	RMSECV (% w/w)
Whole data	Urea	17	0.93	0.22	0.91	0.25
	Glycine	16	0.85	0.78	0.81	0.88
	Taurine	16	0.89	1.13	0.86	1.31
	Melamine	16	0.87	0.21	0.84	0.24
Only Single mixtures	Urea	7	0.92	0.34	0.85	0.48
	Glycine	7	0.89	1.04	0.82	1.33
	Taurine	7	0.91	1.55	0.82	2.20
	Melamine	7	0.89	0.29	0.80	0.39
Only Dual mixtures	Urea	7	0.89	0.25	0.83	0.31
	Glycine	10	0.79	0.76	0.70	0.92
	Taurine	7	0.85	1.21	0.80	1.41
	Melamine	7	0.78	0.25	0.71	0.29
Only Multiple mixtures	Urea	13	0.86	0.17	0.74	0.24
	Glycine	11	0.78	0.55	0.67	0.67
	Taurine	9	0.78	0.91	0.69	1.08
	Melamine	12	0.75	0.16	0.62	0.20

Table 52: PLSR models for adulterated pea protein powder samples scanned with the handheld spectrophotometer through the LDPE plastic bag and analyzed at the spectral range of 950-1650 nm

Model dataset	Predicted adulterant	LV	R^2	RMSEC (% w/w)	R^2CV	RMSECV (% w/w)
Whole data	Urea	13	0.92	0.22	0.91	0.25
	Glycine	16	0.83	0.88	0.77	1.01
	Taurine	17	0.82	1.50	0.77	1.69
	Melamine	16	0.83	0.24	0.78	0.27
Only Single mixtures	Urea	7	0.95	0.28	0.85	0.48
	Glycine	7	0.89	1.04	0.82	1.31
	Taurine	6	0.89	1.70	0.74	2.62
	Melamine	7	0.89	0.28	0.68	0.49
Only Dual mixtures	Urea	7	0.90	0.23	0.86	0.28
	Glycine	7	0.77	0.92	0.68	1.09
	Taurine	8	0.82	1.37	0.73	1.67
	Melamine	7	0.79	0.24	0.74	0.27
Only Multiple mixtures	Urea	12	0.84	0.19	0.77	0.23
	Glycine	12	0.65	0.71	0.34	0.96
	Taurine	11	0.67	1.13	0.52	1.37
	Melamine	11	0.70	0.18	0.52	0.23

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