Rapid monitoring and quantification of unripe banana flour adulteration using visible - near infrared spectroscopy

By

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DECLARATION

I, **Phindile Faith Ndlovu**, declare that the research reported in this dissertation, except where otherwise indicate, is my original work. This dissertation has not been submitted for any degree or examination at any other university.

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RESEARCH SUMMARY

A general lack of strict regulations in South Africa to monitor processed foodstuff increases chances of unfair producers and traders to intentionally mislabel and adulterate high valued food products with inferior lookalikes. Recently, unripe banana flour (UBF) has gained global attention and has been identified as a replacement for cereals flours due to its gluten free traits and resistant starch nutritional qualities, yet has no quality control standards. The objective of this research was to develop rapid prediction models based on a visible to near infrared (Vis-NIR) spectroscopy (Vis-NIRS) combined with multivariate analysis to classify, detect, and quantify different adulteration levels of staple flours (i.e. wheat and maize flours) in unripe banana flour. The other aim was to identify important biomarkers of unripe banana flour that could be used to discriminate unripe banana flour adulteration at different concentration levels. A critical evaluation of the portable Vis-NIR spectroscopy combined with chemometrics analysis indicated that it was possible to discriminate between unripe banana flour with wheat and maize flours and assosciated different adulteration levels. The partial least square (PLS) regression (PLSR) analysis quantified individually maize and wheat flours, based on different adulteration levels, showed that optimal PLSR detection models performances were obtained using the first derivative Savitsky-Golay (7-point smoothing, 2^{nd} order polynomial) and the second derivative Savitsky-Golay (19-point smoothing, 2^{nd} order polynomial). The study to optimise and test the handheld Vis-NIR instruments' feasibilty to simulteniously develop a standard model for rapid solution to detect both maize and wheat flours adulteration indicated high classification and prediction accuracies could be achived through principal component analysis (PCA) and partial least squeres regression (PLSR). The study found that gluten could be utilised as a biomarker to test for unwanted adulteration of unripe banana flour with wheat flour, and showed good and reliable rapid spectroscopic PLSR model was achieved with high precision. Near infrared spectroscopy showed great potential to detect the nutritional changes

of unripe banana flour during adulteration based on resistant starch content. The results of this investigation indicated that wheat adulteration is a threat to unripe banana flour importnt attribute as signification reduction of this parameter was observed with the increasing levels wheat adultearation. Vis-NIR spectroscopy with multivariate analysis detected the varying resistant starch concentration unripe banana flour samples successfully with high accuracy. The results and stability of the models developed in this study demonstrated clearly that the Vis-NIRS method has a potential of providing unripe banana flour processing industry with a rapid and non-destructive technique to manage unripe banana flour quality as well as adulteration by staple flours, therefore ensuring fair and safe trading of the product in retail markets of South Africa.

PREFACE

This thesis represents a compilation of manuscripts where chapters are presented independently. Therefore, some repetitions between chapters have been unavoidable.

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CHAPTER 1 - GENERAL INTRODUCTION

1. Introduction

Fruit-derived powdered food products are highly valued commodities globally, both for food security and health purposes (Chandra and Kumari, 2015). Unripe banana flour, is a product manufactured from a variety of fully mature green banana fruit (*Musa acuminate* and *Musa balbisiana*) (Anyasi et al., 2013). In South Africa, banana flour processing is at a pionner stage and this type of food has recently entered the retail market due to anormous health promoting attributes.

The global banana flour industry is strengthened by a large scale production of different banana cultivars, making the crop available throughout the year. Popularly, in South Africa, bananas are consumed fresh when the fruit is ripe. By contrast, as climacteric fruit, bananas undergo certain changes in the metabolic rate and biochemical mechanism like an increase in respiration and rise in ethylene production accumulate during the process of fruit ripening (Paul et al., 2012). Normally, bananas are harvested fully mature green (i.e. at the pre-climacteric stage). At this phase, the fruit experiences various physicochemical changes such as colour, texture, aroma, nutritional composition and taste (Payasi and Sanwal, 2010). This entail, post-harvest special care is required in order to maintain the fruit fresh for longer periods, in a state acceptable by the industry/market and that also meet consumers' expectations (Zhu et al., 2015).

For producers to keep the banana fruit for fresh consumption, an extra effort need to be taken like using low temperature storages. However, according to Morrelli et al. (2003), bananas susceptibility to chilling injury limit the fruit opportunity to be stored for longer at environments below ambient temperature. Based on the research evidence of these authors, banana fruit could tolerate temperature of 10 ℃ only for a minimum duration of a week prior the initiation of

chilling injury symptoms. However, the use of post-harvest treatments and low temperatures involves relatively higher investment (Mujumbar and Low, 2010). Given over 1200 banana varieties cultivated around the globe, South Africa grows about 70 different banana cultivars of which 20-50% of harvested fruit still go to waste due to the commodities perishable nature (composed of 65-75% moisture content), insufficient post-harvest handling techniques and little knowledge to process the fruit into different forms (Mashau et al., 2012). Some local banana varieties grown in the country are small sized fruit, with unique colours (e.g. Green Red cv.), tastes and unusual curve shapes when compared to dessert cultivars such as Williams, Gros Michel, Chinese Cavendish (Anyasi et al., 2013). Due to this, these local genotypes receive less acceptance both in the local market and don not even qualify for exportation (Kibazohi et al., 2017).

Conversely, cereal/or grain flours nowadays are usually produced from genetically modified organisms (GMOs) in order to fulfill the demand of the food industry (Zhao and Shewry, 2011). In essence, the majority of the functional properties contained in these flours are artificial. The rise in uncommunicable health issues linked with cereal/grain flours has lead modernised consumers and the rest of the public to seek for products containing natural functional properties (for example resistant starch, phenolic compounds, dietary fibres, carotenoids, phytonutrients, ascorbic acid, to mention a few (Khoozani et al., 2019).

Regarding economic, health challenges and banana fruit physiological constrains, the alternative route that banana producers have adopted around the globe is drying the fruit while still at the preclimacteric stage. The dehydration of banana fruit at an unripe phase is an effective industrial technique that most SA producers need to adopt in order to accommodate the fruit surplus, optimise the fruit shelf-life and quality (Karam et al., 2016). Another advantage with this

approach is that products are characterised with low moisture content which also contribute towards product low bulk weight, consequently, relatively low storage and exportation costs are incurred (Da Silva et al., 2014). Therefore, the post-harvest processing of unripe bananas into powdered form is one of the convenient approach to reducing postharvest losses, increase shelf life and availability of the crop throughout the year and preserve nutritional composition (Yani et al., 2013, Bezerra et al., 2013) and also enable farmers, both in commercial and small-scale settings, to diversify on their on-farm business activities (Mohapatra et al., 2011).

It is believed that the production of unripe banana powder and its consumption began in the 1900, where in various parts of Africa and Jamaica society utilised it as a natural gluten-free food sourceand a substitute to cereal-based flours (Ashwar et al., 2016). Unripe banana flour contains good health promoting antioxidants and phytochemical properties which in various research has been proven to reduce degenerative illnesses such as diabetes, colon cancer, obesity (Menezes et al. 2010). Resistant starch type II is the main component, accounting for about 45-75 percent in unripe banana flour (Raigond et al., 2015). Unlike other commonly known cereal and grain flours, the type of resistant starch contained in the banana flour resist hydrolysis in the small intestine and reaches large intestine where it functions as a fermentable dietary fiber feeding the systems microbiota (Sardá et al., 2016b). These health attributes are of benefit for celiac disease sufferers and other patients with many non-communicable diseases (Tavares da Silva et al., 2014). With this, unripe banana flour has gained the attention of nutritional researchers and health conscious consumers. This has also contributed in the accelerated production and marketing unripe banana flour.

A large proportion is used as a primarily ingredient in the food industry which is further segmented into bakery, snacks, confectionery, weaning products (Gumisiriza et al., 2017,

Adeniji, 2015) The global consumer demand for the product has increased market trade as well as its derived products. During the 2014-2018 forecast, global market sales increased by approximately 3.8% compound annual growth rate (CAGR) (Future Market Insights Report, 2018). By 2023, the global banana flour market is estimated to reach USD 537 million from USD 397 million obtained in 2017. Recently, it has been report that for the period 2018-2023 the CAGR is expected to reach 4.2 percent. During this period, the Middle East and African (MEA)countries combined with SA are estimated to reach a market value of USD 300 million. The Latin American countries market value to reach an amount of USD 730 million by the end of 2027 and as the largest banana flour producers, the industry is anticipated to reach an annual compound growth rate of 6.5% towards the end of 2027 (Future Market Insight, 2019).

Standards regarding safety and quality control of agricultural food products states "business manufacturing operations must ensure end product supplied in the retail market is pure, safe and free from contamination and pathogens" (Akkerman et al., 2010). Despite growth in revenues associated with rising consumer demand, the concern with the banana flour market is that, there is a scarcity of technologies put in place to ensure and monitor quality (Sardá et al., 2016a). This gap of information encourages unfaithful processors to cheat the identity of banana flour by adding or substituting it with low value undesired substances. This act is referred to as economic adulteration and is a very difficult case to solve and to distinguish if it happened intentionally as a result of negligence (Manning and Soon, 2014, Khan, 2013).The common underlying reasons for food fraud is usually motivated by greed, high profits linked to the practice; owes to the abundance and easy access to low-cost substitutes and/or artificial illegal matrices with shared homogenous sensory qualities (taste, texture and colour) and chemical characteristics as natural products (Lohumi et al., 2015, Manning, 2016, Thangavel and Dhivya, 2019). As reported by Sardá et al. (2016a), adulteration of unripe banana flour can reach up to 80 percent

with many low-cost edible flours, including wheat and maize flours. The widespread of processed foodstuff adulteration in South Africa is common and has been in the past reported for products such as honey (Downey et al., 2003) and grounded meat (Cawthorn et al., 2013), to mention a few. As suggested in various studies in the literature, outcomes of adulteration practices disrupts business relationships between countries, deteriorates product quality, causes produce and economic loss, and possess health risks (Nasreen and Ahmed, 2014, Rahman et al., 2015, Handford et al., 2016).

The authentication of unripe banana flour is, therefore, a crucial factor in the SA food industry as many consumers and health organisations have become informed about the product benefits. South Africa is one of the developing countries with porous import boarders, and currently the country has one company involved in the banana flour processing (i.e. M-Pak, in Limpopo). Having one company monopolizing banana flour processing means that the industry in SA is still growing, and allows imports of the product from other regions. In order to strengthen the country's competitive edge in banana flour trade, the South African banana flour industry seeks techniques to monitor adulteration acts regarding the product.

Various analytical methods have been employed in the identification of certain product adulteration. These include wet chemistry involving high performance liquid chromatography (HPLC) (Salghi et al., 2014), mass spectroscopy (Azad and Ahmed, 2016) and enzyme linked immunosorbent assay (ELISA) (Doosti et al., 2014, Song et al., 2014). However, the utilisation of such methods is time-consuming and in various literature considered these are insufficient for adulteration detection as they tend to focus or target specific components or compounds during analysis (Haughey et al., 2013).

These analytical methods also give insignificant outputs since food imitators would always be in search for alternative adulterants when a specific adulterant is discovered and included in the list of target analytes (Xu et al., 2015). With that being said, visible-near infrared spectroscopy (Vis-NIRS) is a well recommended non-destructive, rapid and sensitive technique for accommodating the assessment of food adulteration (Zhang et al., 2014, Fu et al., 2014). In this regard, and owing to its simplicity and robustness nature when accompanied by chemometrics modelling, Vis-NIRS is the most suitable tool for qualitative and quantitative evaluation (Cattaneo and Holroyd, 2013, Huang et al., 2016) of banana flour adulteration.

Due to its capability to operate in the region (400-2500 nm) of the electromagnetic spectrum, infrared technologies collect the molecular fingerprints of product under investigation (Cubero-Leon et al., 2014, Riedl et al., 2015), so that the desired quality characteristics and unauthorised added foreign substances in food product can be detected. Each measured fingerprint is then stored as a single wavelength consisting both useful and irrelevant information (Hong et al., 2017). The irrelevant data represented by broad, sharp and overlapping peaks at a later stage during the construction of prediction models are reduced through the application of different preprocessing tools (Teye et al., 2014). The use of visible to near infrared technology has been used extensively in the agro-food industries for the authentication of various products but not yet has it been applied in the process monitoring of unripe banana flour in South Africa. This is also the case for European regions, United states, China, Brazil, India so as the Middle East and West African countries, yet are heavy producers of banana flour and its related commodities.
This research study intends to introduce a new scientific approach that manufactures and retail market operators can use to identify and monitor of banana flour with possible adulteration with relative cheap staple flours. Hence, considerable knowledge and understanding of Vis-NIRS ability to detect less desirable materials (wheat and maize flours) in unripe banana flour is

important not only for economic gain and health reasons but also to ensure fair trade amongst producers.

2. Aims and objetives

The main aim of this research was to develop prediction models based on a Vis-NIRS combined with multivariate analysis to identify quality attribute of unripe banana flour and to classify, detect, and quantify adulteration of levels of staple flours (i.e. wheat and maize flours) in unripe banana flour.

This aim was achieved through the following specific objectives:

- To evaluate Vis-NIRS along with suitable chemometrics tools (PCA and PLSR) to discriminate pure unripe banana flour samples from samples adulterated with wheat flour and build prediction models to quantify different adulterant doses.
- To develop an optimum non-destructive partial least squares regression model to quantify unripe banana flour adulteration with different mixes of maize flour.
- To determine the robustness of the technique with associated chemometrics preprocessing analysis to construct a standard prediction model that can detect both adulterants (wheat and maize flours) various concentrations in unripe banana flour.
- Additionally, exploring Vis-NIRS to identify potential quality attributes that can be used to separate unripe banana flour with staple flours under investigation.

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CHAPTER 2 - A REVIEW OF DESTRUCTIVE AND RAPID NON-INVASIVE METHODS USED TO DETECT ADULTERATION OF DRIED POWDERED HORTICULTURAL PRODUCTS

Abstract

Powdered foods derived from horticultural produce, include spices and herbs, fruit and vegetable flours, hot beverages and medicinal powdered products. They are vital components of the modern human diet and lifestyles. Horticultural powdered products constitute good sensory attributes (flavour and aromas); powerful antioxidants (phenolics, flavonoids, carotenoids); important proteins, starch, minerals, and vitamins, which have specific physiological functions towards improving human health. An exponential rise in the incidences involving adulteration of processed foods is encountered in many agro-food industries globally. The impacts on consumers' health may lead to allergic reactions, chronic illnesses, and sometimes death. Thus, quality evaluation and the maintenance of natural authenticity of these products is the primary concern. Non-destructive spectroscopic techniques such as visible to near infrared spectroscopy (Vis/NIRS), Fourier transform infrared (FT-IR), hyperspectral imaging and Raman spectroscopy are the fourth industrial revolution methods, preferred over traditional methods, for application in the value addition chain to monitor adulteration of horticultural powdered products. The aim of this chapter is to provide an overview of literature on the potential applications of destructive and non-destructive technologies to assess adulteration with emphasis to powdered horticultural foods. An understanding of food adulteration and adulterants concepts and potential impact on health and agri-food industry is elaborated. Potential applications and technical limitations related to the use of destructive methods assessment of adulterated horticultural powdered food is highlighted. This review also discusses operational principles of the infrared technologies including Vis/NIRS, FT-IR,

hyperspectral imaging and Raman spectroscopy. Given the recent studies conducted on powdered food adulteration, chemometric analysis applications towards developing identification models to effectively determine the extent of adulteration levels of various products are illustrated.

Keywords: Vis/NIRS, Hyperspectral imaging, Raman spectroscopy, Multivariate techniques, Powdered foodstuff

1. Introduction

Post-harvest processing of horticultural produce is a transformation process of material in their fresh state to various forms (Floros et al., 2010). The aim is to reduce postharvest losses, add value to underutilized, 'fall-outs' or 'downgraded' produce, a better shelf-life extension of fruits and vegetables for future utilisation (FAO, 1997; Mhazo et al., 2012; Mlambo et al., 2019). After harvest, many food processing activities are involved in the fruit and vegetable value-adding chain. These include different divisions that start from the production method, packaging and storage, distribution and marketing of products. These activities also involve the maintenance of technologies that guarantees the quality of the product at different stages of the value chain.

In South Africa about 29% of horticultural produce is used in processing, whereas 71% is sold as fresh material. Processed food contributes an estimated 30.5% to SA gross domestic product (Thandisa, 2014; Department of Trade Industry, 2014). With many technologies in the food processing sector, drying is one of the processing methods that is used to develop powdered horticultural commodities (Weaver et al., 2014). Various published research indicates that powdered products are generated from a variety of plant materials, the majority being fruit pulp, leaves, peels, stems, tubers, roots, and pods, to mention a few. Essentially, dried

horticultural powdered foods are developed simple by dehydrating plant parts and milling them into powder (Su and Sun, 2018). Compared to fresh produce, powdered horticultural commodities are concentrated with different health-promoting antioxidants and phytochemicals of which, with constant daily intake, promote a healthy lifestyle and assist in reducing life-threatening illnesses (Sun-Waterhouse, 2011). The natural ingredients potency of powdered horticultural products greatly influences the consumers purchasing perceptions and demand for the product.

In developed and developing countries, dried horticultural foodstuffs are used for pharmaceutical and consumption purposes. Furthermore, these are highly valued products for everyday life as their state enables versatile applications during the preparation of many foods. Several foods are incorporated in the powdered products' category with a wide range of unique flavors, aromas, tastes, and attractive chromophores (Su et al., 2017). In this review, four groups, namely, 1) spices and herbs, 2) fruits and vegetables, 3) hot beverages, and 4) medicinal powdered products have been identified to fall in the category of powdered horticultural products.

Quality evaluation for these foodstuffs is imperative for the recognition of unauthorized addition of ingredients (Su and Sun, 2018). Worldwide, adulteration of powdered foodstuff is a common practice in the agro-processing firms. The success in this act is made possible by the easy accessibility of adulterants which mimic the nature of existing or newly established products (Granato et al., 2018).

One of the critical and crucial concepts in many agricultural sectors is to determine and ensure that food quality is guaranteed. Essentially, processed goods undergo varied complex morphological structure alteration/or modification phases (Kamruzzaman et al., 2015a). Figure 1 gives an exemplary illustration of the necessary steps taken during the preparation of the

South African unripe banana flour. Processes involved in food manufacturing makes the final product bear little or no resemblance from their fresh original form (Weaver et al., 2014) (Figure 1). Additionally, during manufacturing, foods are prone to various types of contaminations, not conforming to food safety and quality standards (September, 2011). The adulteration of food may be intentional or unintentional. Deliberate adulteration is motivated by greed whereby unfair producers substitute closely relates products with aims of maximizing their output and profit margins. Unintentional adulteration often arises due to negligence and improper post-harvest processing of produce. All these cases are summed under one phrase and referred to as economic adulteration (Mohammed et al., 2014, Keim et al., 2015, Lohumi et al., 2015). Food adulteration compromises product quality which as a consequence impact on the health of consumers.

To safeguard product quality and mitigate adulteration of powdered horticultural foods, agricultural researchers, engineers and food scientists around the world have developed expertise and methodologies to verify product authenticity. Recent research studies recommend the use of non-destructive tools such as vibrational spectroscopies (visible to near infrared (Vis-NIR)), fourier transform infrared (FT-IR), hyperspectral imaging, Raman spectroscopy) to evaluate adulterants in powdered foods (López et al., 2014; Ding et al., 2015; Pasquini, 2018). However, there is still a body of literature reporting on the successful applications of destructive methods (immunological assay, deoxyribonucleic acid (DNA) techniques, microscopic methods, electrophoretic procedures and chromatographic methods) for this regard (Dhanya and Sasikumar, 2010; Bansal et al., 2017; Galvin-King et al., 2018).

In the current century, destructive techniques are less desirable for use in the food value chain since they are time-consuming, laborious, require continuous utilization of chemical reagents, and in some instances inaccurate since they tend to be compound-specific (McGrath et al., 2018). There is a limitation of reviews that discusses the use of destructive and non-destructive

methods applications on food adulteration with more emphasis on different groups of horticultural powdered products.

This literature review aims to provide current knowledge on the range of technologies developed for evaluating the adulteration of horticultural powdered foodstuffs. It briefly outlines the basic concept of food adulteration and its related impacts. This review chapter also discusses the basic application principles, challenges and future recommendations of these techniques in improving the security of new and existing product authenticity to the processing industry.

2. Theory/Terminology: Understanding food adulteration, adulterants and its associated consequences in the agri-food industry chain

The adulteration of processed foods has been a universal practice and the first research into it entitled "A treatise on adulterations of food and culinary poison" was documented by Frederick Accum in the 1820s. The author surveyed adulteration and possible contamination of various products, owing to false manufacturing procedures, not in line with the food safety standards and regulations. Identified amongst many, tea, coffee and spices (e.g. pepper) along with sawdust, plaster, lead, copper, were detected as potential non-permitted adulterants used in the agro-food industry to heighten the bulk and used as colour agents mentioned products and other confectionery products. Accum's research exposed many fraudulent agro-processing firms, traders and also suggested methods for detecting food adulteration and contamination, applicable during that era (Accum, 1820; André, 2018).

'Food fraud' and/or 'economical motivated adulteration' (EMA) are interchangeable terms used in the scientific literature referring to adulteration of powdered foods (Ellis et al., 2005;

Manning, 2016a; Hong et al., 2017). Moreover, the collective term declaring extraneous substances found within natural food products, limiting their quality and effectiveness, is called an adulterant (Calahan et al., 2016).

As adopted by the food and agricultural organization (FAO) of the United Nation and in other research publications, adulteration of agricultural products means an illegal phenomenon which involves intentional substitution, or accidental addition of various adulterants to pure products to reduce cost of production and increase sales and profit for those products (Spink and Moyer, 2011; Esteki et al., 2018).

Food is considered adulterated if/when the label/package description (i) does not comply with the content of the product; (ii) false stated products geographic origin; (iii) incorrect declaration of the production methods used during growing (e.g. organic versus conventional process); (iv) untruthful statements regarding product preparation method (this includes the type of machinery used i.e. hot air drying, freeze-drying, vacuum drying, sun drying, etc.) and tampered expiry dates (Scarano and Rao, 2014; Manning and Soon, 2016).

There has been significant economic losses and health risks associated with food adulteration. An annual estimated loss ranging from 10 to 40 billion US dollars has been reported globally to result from food adulteration (McGrath et al., 2018). According to Galvin-King et al. (2018), depending on the toxicity of adulterants, approximately 2 -15% annual profits can be lost due to a single adulteration incident per company/or industry. During the 2003-2004 period, the spices and herbs industry experienced an economic loss of about 481 million US dollar following the adulteration scandal of Sudan dyes in products such as red pepper, chilli powder, turmeric, paprika powder and saffron powder (Tarantelli, 2017; Galvin-King et al, 2018). In 2014, different regions of the United States, Canada and European countries experienced over 675 recalls of cumin and taco spice products, as a result of adulteration with almond, peanut

and tree nuts (Garber et al., 2016). Previous and recent research have reported that the consumption of adulterated food may result in allergic reactions to sensitive individuals (Bock et al., 2001; Everstine et al., 2013), which consequently lead to the occurrence of a public health outbreak in the long run (Esslinger et al., 2014; Tibola et al., 2018). For instance, intake of coffee powder adulterated with tamarind and date seeds powder may lead to individuals contracting stomach disorders (Lakshima, 2012). The swelling of the face may be an immediate allergy effect response after the consumption of turmeric contaminated with yellow chalk powder (Nallappan et al., 2013). Greater than 60% individuals were hospitalised as a result of unknowingly consuming spices adulterated with lead oxide in some regions of India and European countries (Everstine and Kennedy, 2013). It is under such circumstances that the challenges to market powdered horticultural products rises, consumers trust and confidence to purchase the valuable powdered food decline (Johnson, 2014).

3. An overview of destructive tools for adulteration detection of powdered products derived from horticultural produce

Destructive methods in different applications have illustrated their potential to identify and detect adulterants in various categories of powdered horticultural products (Table 1 and Table 2 below). The determination of powdered food adulteration can be performed destructively through the use of (i) chemical /or biochemical methods (i.e. electrophoretic and chromatographic techniques), (ii) microscopic methods (iii) protein antibody based methods (i.e. enzyme-linked immunological assay (ELISA)), and (iv) DNA based techniques (i.e. polymerase chain reaction (PCR)). The fundamental operations of these methods significantly differ, as their abilities to detect adulterants in various food mixtures is sensitive to specific marker compounds (Sørensen et al., 2016).

As mentioned in a recent review by Teye et al. (2013), destructive techniques are good as being tools for laboratory research institutions. This is simply because estimates of product quality using these methods is normally based on a few randomly selected food samples (Grassi and Alamprese, 2018). The main disadvantage with small-scale routine quality analysis system is that in a nutshell, a food product can be contaminated with more than one potential adulterants. In many instances this means there are much higher probabilities to provide insufficient information about the authenticity and adulteration of particular food products (Peng et al., 2017; Ballin and Laursen, 2019); hence, many possible types of adulterated food samples can go to different markets undetected (Sørensen et al., 2016).

Given the complex nature of the food supply chains; food production industries, wholesale suppliers and retail markets are required to make it a priority that before distribution every food item is routinely checked for defaults, are authentic and that they comply with the food safety and quality regulations (Callao and Ruisànchez, 2018). Unless stated otherwise, destructive techniques have become less competent and undesirable for use in in-line/ on-line industrial applications due to several technical issues. They are generally expensive, erroneous, time consuming, laborious, continuously require buying of chemical reagents, and the need for highly trained personnel to carry out the analysis (Manfredi et al., 2015; McGrath et al., 2018). The presence of these challenges prompted a need for a shift to more cost-effective and novel rapid techniques.

3.1. Chemical /or biochemical techniques

The detection of powdered food adulteration by electrophoretic and chromatographic methods is associated with the following powerful profiling techniques *viz* capillary electrophoresis (CE), gas and high-performance liquid chromatography (GC and HPLC) (Toci et al., 2016).

The basic concept underlying the use of these analytical approaches require knowledge on the use of suitable extraction solvent, removal or clean-up of interfering components, separation and selective detection of chemical compounds (García-Cañas et al., 2014; Pérez-Míguez et al., 2016).

Capillary electrophoresis and HPLC enables the detection, identification and quantification of adulterants through profiling a variety of food related molecules (such as proteins, phenolics, amino acids, and carbohydrate compounds, amongst many) with different chemical properties (František Kvasniěka, 2005; Bansal et al., 2017). Pérez-Míguez et al. (2016) reported the determination of non-protein amino acids as quality descriptors in variety of adulterated powders including chive, tomatoes, garlic, onions, and cocoa product samples by CE approach. A report by Nogueira and do Lago (2019) demonstrated the use of CE through the characterization of glucose and xylose as quality markers to detect the adulteration of coffee husks and corn flour in instant coffee powder.

Jham et al. (2007, 2008) employed the HPLC technique to demonstrate that the adulteration of coffee powder (*Coffea arabica*) with maize could be identified by the separation of tocopherol profiles and fatty acid methyl ester profiles. In order to detect the adulterants in coffee matrix, Domingues et al. (2014) reported that the monosaccharides profiles could be used as characteristic components of roasted and ground coffee and their adulterated samples with triticale and acaí by HPLC system. Through the profiling of polysaccharides, Yang et al. (2015) established a method to identify adulterated cocoa powder mixed with several adulterants (cocoa shell powder, chestnut shell powder, peanut shell powder, longan shell powder, starch, wheat flour and pumpkin powder) using HPLC and principal component analysis. In a pilot study, Vadivel et al. (2017) considered piperine as a chemical marker to discriminate between pure black pepper from adulterated samples with papaya seed powder using high performance thin layer chromatography (HPTLC) method. Vandekerckhove et al. (2017) established a

chromatographic approach based on an ultra-high performance liquid chromatography with mass spectroscopy (UHPLC-MS) for the detection analysis of peanut adulteration in chilli pepper powder. Sáez Vigo (2019) analysed polyphenols using high performance liquid chromatography ultraviolet (HPLC-UV) and fluorescence (HPLC-FL) coupled with chemometric methods and was able to identify and quantify almond flour adulteration by peanut and hazelnut flours.

GC has detected the adulteration of powdered products by means of separating volatile or semi volatile natural compounds (Haneef et al., 2013; Bansal et al., 2017). The demonstration of the techniques' capability, combined with mass spectroscopy (GC-MS), to distinguish between roasted coffee and their adulteration mixtures roaster barley have been reported by Oliveira et al. (2009). The profiling of fatty acids by GC coupled with multivariate analysis methods was proven to be an eligible method for testing almond powder adulteration with apricot kernel (Esteki et al., 2017). It was possible to distinguish differences in the phytochemical constituents (such as flavonoids, tannins, sterols, coumarins, lignins, proteins and sugars) of black pepper, and adulterated black pepper powder samples with its adulterant papaya seed powder by GC-MS method (Vadivel et al., 2017). Although electrophoretic and chromatographic techniques are available to differentiate the adulteration of horticultural powdered products, there is limited research studies on their use to detect adulteration of medicinal powders (Table 1). These techniques involve the use of sophisticated instrumentation systems which needs skilled technicians to reconnect. On the other hand, various chemicals involved during the analysis generate chemical waste. This requires careful disposal, which is associated with relatively high additional expenses. As a significant drawback, these approaches are not environmental friendly (Haneef et al., 2012; Domingues et al., 2014; Daniel et al., 2018).

3.2. Microscopic based tools

Several microscopic techniques such as electron, optic, light and scanning microscopy have been used by researchers to diagnose the adulteration of powdered horticultural products. As depicted in Table 1, microscopic methods determine the product that is adulterated with other substances through detailed examination of foods different morphological characters, tissue, cell types and bioactive structural features like proteins, starch, etc. (Ballin and Laursen, 2019). A qualitative identification of starch granule patterns was performed under a light microscopy to detect the authenticity, mislabeling and suspected substitution of unripe banana flour with tuber and cereal flours (Sardá et al., 2016).

Zho and Zhao, (2014) showed the efficiency of using microscopic technique to clearly distinguish the micro-morphology characteristics of pure seasoning powders (cumin, chilli, pepper and mustard powders) from the adulterating substances (starch, plant straws, and monosodium glutamate). Moreover, the adulteration of papaya seed powder in black pepper powder could be identified by careful microscopic examination of fatty oils, oil globules, starch granule, fibres, and different parenchyma cell characteristics (Vadivel et al., 2018).

Serrano et al. (2010) was able to discriminate and identify African potato (*Hypoxis hemerocallide*) from its counterparts including *Artemisia annua* L, and *Guiera senegalensis* by application of light and scanning microscopy. These authors also argued and emphasized on the difficulty to analyze powdered samples compared to fresh samples using the technique. In a review by Pastor et al. (2019) it was also indicated that even though microscopic methods are able to differentiate powdered food products by plant species, it may not enable accurate analysis when a mixture of unknown flours is analyzed. Hot beverage powders adulteration has not been investigated by the microscopic application (Table 1). Other than being expensive, microscopic methods require precise sample preparation done by highly trained staff.

Table 1: Recent applications of electrophoretic, chromatographic and microscopic methods to detect adulteration of horticultural powdered products.

Product category	Potential Adulterants	Potential attribute/s	Detection method	Reference
Spices and herbs				
Black pepper powder	Papaya seed powder	Piperine	HPTLC	Vadivel et al. (2018)
		Flavonoids, tannins, sterols, coumarins, lignins, proteins and sugars	GC-MS	
		Fatty oils, oil globules, starch granule, fibres, parenchyma cell shapes	Microscopy	
Cumin, chilli, pepper and mustard powders	Starch, plant straws, and monosodium glutamate	Stomata, starch and fibre structures	Microscopy	Zho and Zhao, (2014)
Chilli pepper powder	Peanut	Proteins	UHPLS-MS	Vandekerckhove et al. (2017)
Fruit and vegetable flours				
Almond flour	Hazelnut and peanut flours	Polyphenols	HPLC-UV; HPLC-FL with PCA, PLS, PLS-DA	Sáez Vigo (2019)
Unripe banana flour	Wheat and corn flours	Starch granule	Microscopy	Sardá et al., (2016)
Hot beverage powders				
Instant coffee powder	Coffee husk and corn flour	Glucose and xylose	EC	Nogueira and do Lago (2019)
Coffee powder	Maize flour	Tocopherol and fatty acids methyl ester	HPLC	Jham et al. (2007, 2008)
Roasted and ground coffee	*Triticale* and acaí	Monosaccharides	HPLC	Domingues et al. (2014)

Table 1: Recent applications of electrophoretic, chromatographic and microscopic methods to detect adulteration of horticultural powdered products.

Product category	**Potential adulterant/s**	**Potential attribute/s**	**Detection method**	**Reference**
Hot beverage powders				
Cocoa powder	Cocoa shell, chestnut shell peanut shell, longan shell, wheat starch and pumpkin powders	Polysaccharides	HPLC with PCA	Yang et al. (2015)
Roasted coffee	Roasted barley	Aroma compounds	GC-MS	Oliveira et al. (2009)
Almond powder	Apricot kernel powder	Fatty acids	GC with PCA, PCA-LDA, PLS	Esteki et al., (2017)
Medicinal powders				
African potato (*Hypoxis hemerocallide*)	*Jateorhiza palmate*, *Artemisia annua* L, and *Guiera senegalensis*	Starch granule, stomata shape, calcium oxalate crystals	Microscopy	Serrano et al. (2010)

3.3. Protein antibody-based technique

Enzyme-linked immunological assay (ELISA) has been reported as an effective immunological method for screening adulteration in various commercial powdered products. Briefly, ELISA is based on an enzyme/ or a protein that catalyzes a biochemical reaction, in order to detect the presence of an antibody or antigen of interest in a food sample (Asensio et al., 2008). The analytical procedure may be conducted qualitatively or quantitatively. A qualitative ELISA is performed using lateral flow immunosticks or dipstick tests based on a simple positive or negative result of a food sample. Where; a positive result means the product is adulterated, and a negative output depicts that a product is authentic and no adulterant is detected (Cawthorn et al., 2010).

A dipstick test is a very convenient and low-cost procedure since all reagents are in the dry form, and the results could be obtained on site within several minutes (Trantakis et al., 2012). Whilst, a quantitative assay is a lengthy method which involves a series of standard solution which requires dilutions of reagents, multiple pipetting, and incubation steps. The detection of food allergen is measured with specialized equipment (microtiter plate reader) (Cawthorn et al., 2010).

In different research investigations (Table 2), ELISA tests have been mostly applied to evaluate and ascertain that processed powdered food products labelled not to contain residues of allergy causing proteins are authentic and safe (Haraszi et al., 2014; Bustamante et al., 2017; Ballin and Lauren, 2019). For instance, Trantakis et al. (2012) by dipstick test quickly determined the authenticity between Arabica and Robusta coffee species and could discriminate 5% adulteration of Robusta coffee in Arabica coffee powder samples.

Sletten et al. (2005) showed that the ELISA could be used as a method to verify inadequate allergen labelling and detect casein (a cow milk protein of which some consumers are

intolerant) traces in products such as instant potato flour and spice mix foodstuffs. Moreover, the adulteration of several products including cocoa powder, hazelnut, and walnut flours with abrin (an allergen protein in beans of *Abrus precatorious* species) was detected by Garber et al. (2008) through this technique.

Moreover, Sharma et al. (2014) preformed a market survey using various ELISA kits to determine the safety of different horticultural food categories (including: tapioca starch, dried cranberries, chilli curry powders, tomato and mushroom soup mix, quinoa, amaranth, almond and coconut flours) and their compliance with gluten free labelling. Different walnut-based powdered products (including sesame and jujube powders) with no label content of walnut proteins, were identifiable through the use of indirect ELISA method (Fang et al., 2015). Vandekerckhove et al. (2017) verified several chilli pepper powder samples to contain undeclared allergenic peanut traces by ELISA analysis.

Although several studies have utilised this technique with success, some research have found this method to be producing false positive or false negative results. As previously mentioned, ELISA approach mainly targets protein allergens towards identifying different products adulteration. However, due to different thermal processing techniques, particular proteins may be denatured during industrial processing of raw food materials (Asensio et al., 2008). Consequently, protein strands of interest may not be present in the condition detectable by the antibodies or antigens of the assay, thus leading into possible errors and inconsistent findings (Manfredi et al., 2015; Prado et al., 2016).

Prior ELISA analysis, it has been reported that powdered food products may require extensive purification in order to eliminate cross-reactivity effect of antibodies with other proteins in the food sample mixture and improve on the techniques' sensitivity and accuracy to detect the adulterated products (Dhanya and Sasikumar, 2010; Fang et al., 2015). The lack of sensitivity

of several ELISA methods to determine undeclared peanut proteins in a variety of cumin spice samples have been recently reported by Garber et al. (2016). The generated inconsistencies in their findings demonstrate the disadvantage of using single analyte-specific assays. These authors emphasized on the need to use multiplex techniques and have alternative analytical methods to address and/ or detect incorrect labelling of allergens in food powders, even if present at trace levels.

3.4. DNA based techniques

Adulterants detection using DNA methods are mainly performed using polymerase chain reaction (PCR) assay procedures. DNA testing procedures are based on the extraction of short or whole sequence of target biological genes, and amplifying those genes for further molecular analyses using various PCR primers (Lockley and Bardsley, 2000; Dhanya et al., 2008, Bansal et al., 2017). Different PCR approaches (Table 2) that have been proven robust to detect the adulteration of powdered horticultural foodstuffs include random amplified polymorphic DNA (RAPD), sequence characterized amplified regions (SCAR), DNA barcoding and real-time PCR, among others (Dhanya and Sasikumar, 2010).

RAPD assay is a simple, low cost, and a quick procedure. It does not require previous sequence information, and this gives it an ability to detect different varieties of plant based adulterants (Bansal et al., 2017; Galvin-King et al., 2018). Using RAPD primers, Dhanya et al. (2010) reported successful detection of the adulteration of chilli powder with its adulterants dried red beet powder, almond shell dust and powdered jujube (*Ziziphus nummularia*) fruit. Even though RAPD is simpler and allows the detection of different adulterants, its main problem is that it lacks sensitivity whenever a change in experimental conditions occurs. This may result into inconsistent findings when the analysis is repeated (Dhanya and Sasikumar, 2010).

SCAR-PCR assay is an alternative of RAPD, it facilitates sensitive and specific screening of adulterants in commercial powdered products. Studies that have demonstrated the potential use of SCAR-PCR include the detection of cashew husk in dry tea samples (Dhiman and Singh, 2003); differentiation of pawpaw seeds in black pepper (Sasikumar et al., 2005; Khan et al., 2010); and discrimination of saffron from safflower herbs (Javanmardi et al., 2011).

Other research studies have used DNA barcoding technique to authenticate and detect the substitution between products derived from the same genus which differ in quality grades (Lockley et al., 2000; Newmaster et al., 2013). The technique has been reported by Parvathy et al. (2015) as a method that can be used to discriminate between different turmeric powder varieties. These authors also observed that this was a potential method to identify the molecular variability in the turmeric powder samples caused by the presence of undeclared plant-based traces of cassava, wheat, barley and rye starches. Moreover, DNA barcoding was proven to be an efficient tool to discriminate the diversity between related spices and herbs (thyme, turmeric, basil, ginger, cardamom, and anise powders) and was shown to be able to identify wheat and rice genes in adulterated ginger powder and milled thyme medicinal herbs (Mosa et al., 2018).

However, it is a challenge to possibly use DNA barcoding for a routine quality analysis as one requires to have in place a database of species DNA sequences as a reference (Mattia et al., 2010). Real-time PCR is another effective DNA method that recently have been acceptable for authentication and adulteration evaluation of powdered horticultural foodstuffs. Alary et al. (2007) developed chestnut specific primers to detect possible presence of cereal (such as common wheat, durum wheat, maize, barley, oat, rye and rice flours) and leguminous (including: kidney bean, soybean, chickpea and flava bean flours) species adulteration in chestnut flour. Sanchiz et al. (2020) also developed a real-time PCR based method to detect hulled wheat flour in chestnut flour of Miquelenca variety.

Compared to other PCR methods, real-time PCR method is highly quantitative since it can simultaneously characterize DNA sequences and detect undeclared adulterants even if the DNA strand has experienced alteration and fragmentation (Bansal et al., 2017). The unique DNA and its stability across plant species have made DNA based techniques deliver reliable and efficient results for the detection of a vast range of adulteration involving different horticultural products (Habza-Kowalska et al., 2019). However, challenges associated with cost as well as the requirement to have highly trained stuff to run the analyses and interpret results limit the use of DNA methods in food industry for routine quality analysis (Sheikha, 2019).

Table 2: Applications of antibody and DNA based techniques to identify adulterants in horticultural powder products.

Product type	Potential adulterant/allergens	Detection test kit/primer	References
Spices and herbs			
Chilli curry powder	Gluten - wheat, rye and barley cereal flours	R7001 Sandwich ELISA	Sharma et al. (2015)
Cumin, Taco spice	Peanut and almond protein	Veratox ELISA xMAP Multiplex ELISA	Taylor et al. (2015); Garber et al. (2016)
Chilli pepper powder	Peanut protein	R6202 Sandwich ELISA	Vandekerckhove et al. (2017)
Spice curry powder mix	Casein - milk protein	Competitive ELISA	Sletten et al. (2005)
	Ovalbumin - egg protein Casein - milk protein Gluten - wheat flour Peanut	M2101, M2102, M2103 and M2104 Quantitative ELISA	Surojanametakul et al. (2012)
Chilli powder	Dried red beet powder, almond shell dust and powdered jujube	RAPD - PCR	Dhanya et al. (2010)
Black pepper	Pawpaw seeds	SCAR - PCR	Sasikumar et al. (2005); Khan et al. (2010)
Saffron	Safflower	SCAR - PCR	Javanmardi et al. (2011)
Turmeric powder	Cassava, wheat, barley and rye starches	DNA - barcoding	Parvathy et al. (2015)
Ginger powder and pulverized thyme	Wheat and rice species	DNA - barcoding	Mosa et al. (2018)
Fruit and vegetable flours			
Instant potato flour	Casein - milk protein	Competitive ELISA	Sletten et al. (2005)
Hazelnut and walnut flours	Abrin - *Abrus precatorious*	Poly-poly Sandwich ELISA and Poly-mono Sandwich ELISA	Garber et al. (2008)
Sesame and jujube powder	Walnut protein	Indirect ELISA	Fang et al. (2015)

Table 2: Applications of antibody and DNA based techniques to identify adulterants in horticultural powder products.

Product category	Potential adulterant/ allergens	Detection test kit/primer	References
Fruit and vegetable flour			
Chestnut flour	Cereals - wheat, durum wheat, maize, barley, oat, rye and rice flours Legumes - kidney bean, soybean, chickpea and flava bean flours	Real-time PCR	Alary et al. (2007)
Tapioca starch, dried cranberries, tomato, mushroom, quinoa, amaranth, coconut and almond flours	Gluten - wheat, rye and barley cereal flours	R7001 Sandwich ELISA	Sharma et al., (2015)
Chestnut flour	Spelt wheat flour	Real-time PCR	Sanchiz et al. (2020)
Hot beverage powders			
Tea	Cashew husk	SCAR-PCR	Dhiman and Singh (2003)
Cocoa powder	Abrin - *Abrus precatorious*	Poly-poly Sandwich ELISA and Poly-mono Sandwich ELISA	Garber et al. (2008)
Arabica coffee	Robusta coffee	Dipstick test	Trantakis et al. (2012

4. Fundamentals of non-destructive spectroscopic methods

4.1. Theory and principle of operation

In principle, non-destructive methods are an evolution from destructive wet chemistry laboratory analysis. Their rapidity and robustness contribute to detect, monitor and prevent problems arising from the production processes of several kinds of powdered foodstuff which may, if unnoticed, result in noncompliance with product specification and food quality standards (Esteki et al., 2018). Non-destructive techniques inspect or evaluate characteristics of food material without affecting or hampering its biological components and potency (Black et al., 2016). In addition -, these tools enable the manufacturer and everyone within the food value chain to ascertain that the final product does not have any hidden defect. Product quality can be determined through several non-destructive techniques. Among spectroscopic tools, visible-near infrared/mid-infrared (Vis-NIR/MIR) spectroscopies; Fourier transform infrared (FT-IR), hyperspectral imaging and Raman spectroscopy are most used methods in the agro-processing industry to address issues of adulteration and authenticity of powdered commodities (Nawrocka and Lamorska, 2013).

Ideally, near-infrared spectroscopy operates in the range between 400-700 nm (visible range) and 700-2500 nm (near-infrared range) of the electromagnetic spectrum (Ding et al., 2015). The light received by food material, is absorbed/reflected/transmitted back to the instrument detector. The interaction between the instruments electromagnetic radiation and the food matrix results into an NIR spectrum which is a reflection of that particular products composition (Haughey et al., 2015).

Generally, NIR spectra is characterised by very weak and sharp imposed absorption bands (Lohumi et al., 2015). This makes the visual evaluation of useful information by the naked eye impossible. The broad peaks of NIR spectra for food material correlate with overtones and

combination bands of carbon-hydrogen (C-H), oxygen-hydrogen (O-H), nitrogen-hydrogen (N-H), sulphur-hydrogen (S-H) chemical molecules (Nicolai et al., 2007). Briefly, overtones result from vibrational excitations of molecules from the ground state to higher vibrational energy levels. A combination is generated when two or more vibrational modes of food materials simultaneously get altered or excited (Rébufa et al., 2018; Mishra et al., 2018).

According to the literature, molecular bonds universal in biological food matrixes relate to carbohydrates (C-H), proteins/or lipids (N-H), water (O-H), and natural pigmentation structures (C=C, C=O) (Fu et al., 2017; Rodríguez et al., 2019). Moreover, in dehydrated food material, C-H, N-H, O-H strongly absorb in the near-infrared region (700-2500 nm). Whereas pigmentation structures dominate the visible region (400-700 nm) and relate to conjugate and aromatic ring structure, not limited to carotenoids, anthocyanins, of powdered foodstuffs (Magwaza et al., 2016; Su and Sun, 2018; Peleng et al., 2019).

The bands noticed in the Vis/NIR region of the electromagnetic spectrum are usually accompanied by unnecessary noise and baselines shifts that hide the useful spectra. The irrelevant data may result either from the instrument scattering effects, noise, temperature effects and/or products changing molecules after being illuminated by incident light from an instrument (Lohumi et al., 2015). Consequently, it becomes a challenging task to view absorption peaks and assign molecular compounds of food materials (Tao and Peng, 2014; Pasquini, 2018). The information acquired in the form of spectra/or images acts as a fingerprint that allows for the identification of distinct molecular characteristics of powdered foodstuffs (Cozzolino, 2014). Food analysists then make use of that fingerprint to differentiate products according to their authenticity without the repetitions of laboratory extraction procedures (Alender et al., 2013).

4.2. Infrared (IR) Instrument specification and setup

The commercial non-destructive spectrophotometer operates at various wavelength regions. Data of a food material could be processed using a visible to near-IR (NIR) (400-2500 nm); mid-IR (MIR) at a region of 2500-15000 nm, and far-IR (FIR) at 15000-10000 nm (Gosh and Jayas, 2009). In addition, infrared methods are equipped with various system configurations. Briefly, a spectroscopy consists of a reference tile, sample compartment, lens/light source (such as a tungsten-halogen lamp), detector (e.g. indium-gallium-arsenide (InGaAs), photodiode, lead sulphide and silicon detectors), wavelength selection setup option and spectra signal processor/ computer system (Cozzolino, 2014).

However, portable infrared spectroscopies design slightly differs, depending on a brand, they are battery-operated, have fixed wavelength setup, which could start from (285/350 nm) visible region (1200 nm) short to the near-infrared region (Peleng et al., 2019; Teye et al., 2019). On the other hand, the stable benchtop instruments are designed with full wavelength region (400-2500 nm), which then gives the operator an opportunity to choose the region they want to base their analysis (Pasquini, 2018). Handheld and benchtop visible to near-infrared spectroscopies, and other NIR based methods, are light powered integrated systems. They send particles of light into the product. From there, the projected IR radiation interacts with the external and internal molecular components of materials that are later used to quantify the intended quality characteristics of foods selected by a researcher (Nicolai et al., 2007).

5. Mathematical methods used for adulteration classification and quantification

An ancient drawback of non-destructive tools has been the issue of not being able to immediately and directly allocate discrimination wavelength regions where the constituents of

the adulterant and natural product interact with light in the electromagnetic region. Various factors could affect the spectra of powdered food matrices during the data analysis and model establishment. Such factors include particle size distribution of samples, chemical bonds and molecular interactions complexity between materials, environmental conditions inconsistent, noise, baseline drifts to mention a few (Nicolai et al., 2007; Magwaza et al., 2016; Quelal-Vásconez et al., 2018).

The theory of operating NIR devises for adulteration evaluation of powdered products is based on the spectroscopies conjunction with multivariate analysis methods. Specialised essential mathematical/or statistical tools, which come as software packages called chemometrics techniques, are implemented to spectral data to identify relevant information and achieve useful model predictions of adulterants concentrations in powdered food matrixes of interest. Moreover, multivariate analytical methods ability to predict adulteration level between commodities works better when their application is examined through the use of pre-processing tools. The most practically used chemometrics and pre-treatment methods in the analysis of powdered horticultural food are illustrated (Figure 1), respectively.

5.1. Multivariate analysis methods

Multivariate analysis is an approach to visual NIRS data, enables the management, identification and understanding of patterns in a large spectroscopic raw dataset (Magwaza et al., 2016). Two approaches of chemometrics analysis exist for the evaluation of adulteration in powdered food samples i.e. unsupervised classification and discriminant methods and supervised regression procedures (Figure 2).

Multivariate unsupervised methods are exploratory tools used to link the relationship between samples and the concentration gradient of adulterants. During the process to screen for

adulteration, information about samples disseminates whether the product is natural (pure/authentic/ or unadulterated) and shows a trend in sample distribution concerning the variation in adulterant concentrations. In this regard, principal component analysis (PCA) and hierarchical cluster analysis (HCA) are the most commonly used unsupervised chemometric exploratory tools when the objective is to classify/ or group powdered products based on purity, adulterant dilution level, production regions and processing methods (Cebi et al., 2017).

In many near-infrared spectroscopic data analyses, PCA is the first step applied to map and describe spectral data patterns, and that many analysts used as the go-ahead before the development of a calibration regression models. For PCA classification, correlated spectral data is digested into a set of principal components (PCs) holding meaningful interpretable results about the explained variance between products under research. Ideally, principal component one (PC-1) explains the larger portion of the variance in studied samples and the subsequent PC-2 will explain the remaining variance not accounted in PC-1, and so on.

Multivariate supervised methods aim to find the best linear combination relationships between the spectroscopic data and the reference concentration of an adulterant (Despagne et al., 2000). Partial least squares regression (PLSR); multivariate linear regression (MLR), partial least squares linear discriminant (PLS-DA), soft independent modelling of class analogy (SIMCA) are all quantification techniques used for modelling and developing adulteration prediction regressions. PLSR is the most powerful modelling method used to build calibration/prediction models to evaluate adulteration. The choice on which chemometric approach to use depends on the researcher and the type of experimental data at hand.

Several aspects must be taken into consideration for a successful classification or PLS regression model. A good NIR model relies on the suitability of a set collected to train the model (Xu et al., 2015). A reference method is required to characterise useful features of the

authentic product to obtain a distribution of samples and to distinguish the unadulterated and adulterated product. However, before that, through the implementation of a chemometric software, raw data is organised in rows and columns matrix. Where the rows represent adulterant concentrations (Y) and the column contains visible-near infrared spectroscopic wavelengths (X). An NIRS regression model is constructed based on dividing the experimental spectroscopic dataset into two sets. The first dataset is utilised as the training/ and or calibration set. It is recommended that the dataset assigned to develop the calibration/ and cross validation model consists of at least 70 or 75% of the collected spectral data and reference lab data of the adulterant content. However, this percentage value is determined by how spread out is the representative sample datasets. This dataset is all used to develop the calibration prediction model (Bagchi et al., 2016).

The first important step to analysing Vis/NIRS data is to learn the spectra for areas of potential noise and baseline shifts. This step of data handling assists in excluding irrelevant and redundant wavebands not efficient to develop a robust prediction model (Ge et al., 2011). The practical role of wavelength deletion selection is to view the appropriate NIR region explaining the characteristics of the studied products and to avoid overfitting the modelling analysis. The second step is to search for spectral outliers which are a hindrance during regression analysis. Outliers can overestimate or underestimate the developed model. Therefore, their removal is pivotal for reliable adulteration assessments. Lastly, a validation method that will test/validate the developed model based on new measured variables (Riedl et al., 2015).

The second data selection is the validation or test set data which usually consists 30 or 25% of the collected spectroscopic and lab reference values and is used to test the models for future stability (Paradkar et al., 2003). In some situations, an adulteration prediction model is developed by doing a 50% data split of the obtained dataset (Contal et al., 2002). An appropriate adulteration detection model can be constructed with various latent variable

selection procedures, i.e. leave one out cross-validation method or a test set validation method. The former, also known as full cross-validation, estimates the statistic on the performance of a predictive model while testing the models' ability to predict new data not included in the calibration set. The later compute the prediction model by interchanging calibration and validation/test set samples during model development (Despagne et al., 2000; Yang et al., 2013).

5.2. Pre-processing techniques and their application to detect powdered products adulteration

The proper implementation of a pre-processing method is a necessary step towards improving spectral data quality and highlight signal differences caused by the contents of adulterants. Moreover, the pre-processing methods are applied to spectral data in order to exclude unwanted spectral noises, to improve waveband selection of NIR characteristics, and the models overall performance (Duchesne et al., 2012). Different chemometric pre-processing measures are evaluated individually or in combination during the research assessment of adulteration, each with specific functions. Common pre-processing methods include smoothing, normalization, derivatives (first and second), baseline correction, standard normal variate (SNV), and multiple scatter correlation (MSC) Figure 2. Smoothing and derivative methods interpret spectral data by drawing out noise through the application of Savitsky-Golay logarithm and gap filters (Næs et al., 2004). The first and second derivatives are responsible for removing noise and improving on the quality of spectral resolution (Savitsky-Golay, 1964). Normalization aims to reduce baseline variation (Downey et al., 1997), while MSC and SNV minimise scattering effects caused by sample particle size effects (Dhanoa et a., 1994).

In many Vis-NIR studies, researchers have evaluated the effect of different pre-processing methods on the development of applicable quality control models that can possibly predict the adulteration of various horticultural powdered products. Liu et al. (2013) evaluated five mathematical pre-treatments (baseline correction, normalization, smoothing, 1st and 2nd derivatives (Savitsky-Golay) at 5, 9 and 13 different point gaps) to investigate the use of mid-infrared Fourier transform spectroscopy for the determination of potato and sweet potato starches in lotus root powder. From this study it was observed that pre-processing spectral with 1st derivative (Savitsky-Golay) with 9-point gaps, smoothing, baseline correction and normalization resulted in 99% optimised adulteration PLS prediction models than the other pre-processing methods. Working on a similar investigation, Xu et al. (2013) using NIR spectroscopy with Savitsky-Golay Smoothing, 2nd derivative and SNV pre-treatments studied the adulteration of starches from cassava, maize, potato and sweet potato in lotus root powder. The authors observed that the SNV pre-processing method resulted in superior SIMCA and partial least square class model (PLSCM) predictions with 93% accuracy compared to other pre-treatments.

Five types of data transformation methods (mean centering, MSC, SNV, first and second-order derivatives) were employed by Hu et al. (2018) to discriminate sorghum and Sichuan pepper in black pepper powder. In this study, accurate and reliable FT-IR classification models using PCA, PLS-DA and genetic optimized support vector machine (GA-SVM) obtained in the range 400-4000 cm^{-1}, showed mean centering as the optimal pre-processing (PCA = 80.7%; PLS-DA = 98% and GA-SVM = 98%) for all classification models. Orrilli et al. (2019) performed a PCA, SIMCA and PLSR analysis to investigate NIR hyperspectral imaging for the detection of black pepper adulteration with grounded papaya seeds. The authors evaluated individual pre-treatments (1st and 2nd derivatives (Savitsky-Golay), SNV, MSC, OSC) as well as combined pre-treatments (SNV+1st derivative (Savitsky-Golay), SNV+2nd derivative

(Savitsky-Golay), OSC+1st derivative (Savitsky-Golay), OSC+2nd derivative (Savitsky-Golay) and Smoothing (Savitsky-Golay) +2nd derivative (Savitsky-Golay). Observations from this study showed that pre-processing with SNV+2nd derivative (Savitsky-Golay) resulted in better PLSR models (R^2 = 0.930; RMSEP = 2.51%) than models developed with one pre-treatment; while Smoothing (Savitsky-Golay) +2nd derivative (Savitsky-Golay) pre-processing achieved 86% and 100% classification accuracy for PCA and SIMCA models, respectively.

Using NIR spectroscopy (1100-2500 nm), Quelal-Vásconez et al. (2018) applied a combination of two pre-processing techniques (second derivative Savitsky-Golay Smoothing+orthogonal signal correction (OSC)) to identify and determine pure cocoa powder in a mixture adulterated with carob flour. The authors obtained good classification and prediction models, showing PCA model with 91% explained variance, PLS-DA model with 100% classification and PLS prediction of 0.97 (R^2) and RMSEP of 3.2%. A follow up study on the fast-screening detection of cocoa shells adulteration in cocoa powder was performed by the same group of researchers. Under uniform NIR technique specifications, a total of seven PLS models were constructed with the extended MSC, SNV, 2nd derivative (Savitsky-Golay), OSC, including combination of all with OSC. The results from this research showed that pre-processing spectra with extended MSC+OSC gave PLS-DA classification of 74% and superior prediction model (R^2 = 0.97; RMSEP = 2.43%) than the other pre-treatments (Quelal-Vásconez et al., 2019).

Deduced from these studies, it can be collectively stated that there has been no rule of thumb which clearly states when to avoid or make use of certain pre-processing methods. The diversity amongst pre-processing methods makes it difficult to conclude on having only one pre-treatment, exclusively designed to fulfil all adulteration determination across different categories of powdered horticultural products (Rinnan et al., 2009; Engel et al., 2013; Horn et al., 2018). However, the accuracy of a pre-processing method is mostly based on its capability to remove unwanted spectral variation while retaining the spectral features that enables the

recognition between authentic and adulterated samples (Hu et al., 2018; Horn et al., 2018). This argument is further supported by observing maximum explained variance percentage (for classification model), and lowest error of prediction and highest R^2 for the external dataset (for quantitative prediction model).

5.3. Measuring adulteration through regression model and statistical criterions

The regression models accuracy is usually measured through the application of equations and indexes. Drawn from trends of various research, depicted in Table 4 are global statistical parameters for assessing the performance of the adulteration prediction model. These indices are a set of mathematical simplified equations suitable to adequately explain, describe the accuracy of the prediction model, give the sensitivity of the NIR spectroscopy and also differentiate where the model can be applied in practical situations (Lohumi et al., 2017). Optimal NIR regression models are usually evaluated based on the values of the coefficient of determination/ correlation coefficient (R^2), root mean square errors (RMSEs), residual predictive deviations (RPD), the range error ratio (RER) and bias. R^2 measures the accuracy of the proportion of explained variance of the response variables both in the calibration and validation or prediction model. As a general rule of thumb, an appropriate regression model to identify and predict adulteration in pure products is the one showing high R^2 values that are closest to one as possible. Concisely according to Williams (2003), R^2 is the percentage of the variance in Y variable (measured reference value) that is accounted for by the X variable (Spectra). A model resulting in a value between 0.5 and 0.65 depicts that more than 50% of the variance in Y was accounted by the variability in X to discriminate between sample concentration, while R^2 values between 0.66 and 0.81 means an appropriate prediction quantification, the models with R^2 values between 0.82 and 0.90 indicate good prediction models whilst calibration models with R^2 values above 0.91 are excellent models.

RMSE measures the sensitivity of the model and these values must be lower than the R^2 values (Magwaza et al., 2016). RPD, the dimensionless ratio of the standard error of prediction to the standard deviation of lab measured reference values. It represents models applicability using three modes i.e. RPD values less than 1.5 are considered unreliable, those between 1.5 and 2 are considered good for rapid screening in breeding programs, while RPD values above 3 are excellent models for product quality assessments (Davey et al., 2009). Another statistical parameter to evaluate the goodness of fit of the calibration model is the range error ratio (RER). According to Yasmin et al. (2019) RER is calculated as a ratio of the difference between the maximum and the minimum reference values in the prediction set. The RER value close to or greater than 10 is an indication of a good model (Williams and Norris, 2001; Yasmin et al., 2019). Bias, is another statistical parameter for evaluation of regression model performance and this parameter should show the low average difference between predicted and measured values for good model accuracy (Bellon-Maurel et al., 2010).

6. Applications of vibrational spectroscopies in agri-food industry for adulteration assessment of horticultural powders

6.1. Vis-NIR techniques

Vis/NIR spectroscopies have demonstrated potential in the agro-processing industry to address the adulteration and authenticity of powdered commodities (Table 3). The technique is based on either the reflectance, absorption and transmittance modes of data acquisition. The spectrum acquisition is obtained over the entire NIR region range (400-2500 nm) (Esteki et al., 2018). The application of NIR spectroscopy coupled with PCA, and radial basis function-partial least squared (RBF-PLS) distinguished successfully between pure purple sweet potato powder from white sweet potato adulterated mixtures (Ding et al, 2015). A portable micro NIR spectroscopy

was used to identify and quantify adulterants (corn peel and corn stocks) in Robusta coffee using PCA and PLS and gave a limit of quantification range of 5-8% and 92-98% prediction accuracy (Correia et al., 2018). Using NIR spectroscopy, adulteration of chilli powder with Suden dye I was evaluated through PCA and PLS-DA and the resulted model gave 0.25% limit of detection (Haughey et al. 2015). Recently, the adulteration of unripe banana flour was evaluated using a handheld Visible and near-infrared spectroscopy, through PCA and PLSR analysis. In this study, PCA resulted in a 95% classification of the variability between pure unripe banana flour from samples having wheat flour adulteration levels while, PLSR was able to give a 99% (R^2_p) prediction accuracy and RMESP of 1.993 g/kg (Ndlovu et al., 2019). Moreover, rosin powder, cornflour, wheat and rice bran using NIRS and PLSR were identified as potential adulterants in Sichuan pepper powder, resulting in determination coefficients of prediction (R^2_p) range 95-99% and standard error of prediction (SEP) of 1.1-3.2% (Wu et al., 2017). From previous research on visible and near-infrared spectroscopy, the technology has demonstrated the feasibility to undoubtedly be used by food industries to guarantee quality for a variety of powdered products.

6.2. Fourier-transform infrared (FT-IR)

FT-IR is another set of near-infrared instruments commercially used for detecting food adulteration. Their potential has been demonstrated with success in different product adulteration research (Table 3). These technologies are an improvement of Vis/NIR spectrometers and they operate under a similar principle (as Vis/NIRS explained above). However, FT-IR instruments after taking the measurements of food sample, the detector converts the radiated electromagnetic energy to a food matrix and represents it as an interferogram instead of a spectrum (Su and Sun, 2018). The use of Fourier transform mid-infrared spectroscopy investigated by Hu et al, (2018) both identified adulteration of sorghum

and Sichuan pepper and examine the origin and authenticity of 150 black pepper samples and for GA-SVM and PLS-DA calibration and prediction models achieved 100% accurate classification rate.

By using synergy interval PLS (siPLS), models with an improved performance developed using FT-IR spectroscopy, demonstrated detection limits ranging from 1.0% to 3.1% (w/w) during the authenticity of saffron with various plant derive adulterants (Petrakis and Polissiou, 2017). Fourier transform infrared (FT-IR) spectroscopy made it possible to detect corn starch adulteration in onion powder and demonstrated PLSR model prediction (R^2_p) of 0.90 and standard error of prediction (SEP) of 3.12% (Lohumi et al, 2015). Corn and barley in roasted and ground coffee were detected using FTIR spectroscopy coupled with linear discriminant analysis (LDA) and the classification models gave 100% prediction being able to predict levels of adulteration as low as 1% (Reis et al., 2013).

6.3. Hyperspectral imaging

As for hyperspectral imaging spectroscopy, the acquired information about food material involves the fusion of images and NIR spectroscopy. It is characterised by remote sensing abilities. Hence, during the analysis, this technique identifies and maps adulterated powdered food without any physical contact (Duchesne et al., 2012, Esteki et al., 2018). Chinese tea (green, yellow, white, black and oolong tea) were classified according to brand for authentication purposes using hyperspectral imaging and a 98.4% prediction accuracy was obtained with library support vector machine (Ning et al., 2017). For quality control, herbal tea (rooibos, honeybush, buchu and cancer bush tea) were successfully separated in terms of categories with hyperspectral imaging (Kiani et al., 2018).

6.4. Raman spectroscopy

The Raman spectroscopy, a more advance approach for adulteration inspection since measurements of a biochemical composition containing important information can easily be explained through observing on a computer system (Li et al., 2015) and without pre-processing, even if they contain the same attributes in different arrangement bands (Yaseen et al., 2017). Sudan dye I-IV are common adulterants detected in many fraud incidents of spices, usually used as agents to enhance the appearance, increase bulk and market sales. Raman spectroscopy was investigated to detect chilli powder adulteration with Suden dye were discriminated through applying PCA and PLS-DA, the resultant models gave the limit of detection of 0.88% (Haughey et al., 2015).

6.5. NIR spectroscopy research and applications to horticultural powdered foodstuff adulteration and quality analysis

Rapid technologies have a range of applications in the food processing industries and scientific research institutions (Huang et al., 2008). These methods have been used for authenticity analysis for a variety of processed products including fruit puree, honey, milk powder (Paradkar et al., 2003; Yang et al., 2013; Kamruzzaman et al., 2015a) among many. NIR spectroscopies have the feasibility to differentiate adulteration and assess the authenticity of products such as spices and herbs, hot beverage powders (i.e. tea and coffee), fruit and vegetable-derived flours and medicinal powdered products. The recent trends of research and applications of infrared methods are illustrated in Table 3 below. Over five years, hyperspectral imaging is the most adopted method to online quality determination, safety and authenticity of tea and coffee products. Its application has been suitable during verification of various product brands, sensory appearance, identifying geographic traits and type of preparation method used (Liu et al., 2017). There has also been a deficit of recent research conducted with Raman

spectroscopy assessing fruit and vegetable flour adulteration. It can be attributed from recent literature that Vis-NIRS and FT-IT are the most commonly researched methods for the monitoring of powdered horticultural product adulteration (Table 3). Even though in the South Africa context, it can be argued that little research or investigations have been made on the subject under review (adulteration monitoring) with more attention to powdered products. However, evidence on other literature shows that adulteration monitoring of spices (September, 2011; McGoverin et al., 2012) as well as fruit derived flour (Ndlovu et al., 2019) and other processed products (outside scope of this review) such as honey (Downey et al., 2003) meat has been scientifically reported (Cawthorn et al., 2013, Payne, 2019) using the novel non-destructive techniques.

7. Challenges and future remarks

By looking at the gap between when adulteration problems started (the early 1800s till today), a variety of methods have been continuously designed and tested to be good to evaluate and inspect adulteration in agro-food industries. Having said that, despite new and evolutional technology one can hypothesize that adulteration is a food quality problem that is not going anywhere. The assumption that expensive food on the market is nutritionally most superior has been for the longest period circulating as a false perception that consumers use to judge product integrity (Charlebois et al., 2016). The moment an adulterant, through research, is identified and a proper technique to evaluate it is established, eventually the perpetrators/unfaithful producers already seek out the next low-price lookalike material they can use (Kar et al., 2018). Food quality control researchers are thus challenged to keep up with learning and predicting new potential adulterants on the market. This to consumers is a concern because scientific research discoveries/ or information regarding an adulteration event of specific foods becomes available post-illness diagnosis. Research progression regarding adulteration issues occurrence

is evidence that food manufacturing industries nowadays depend on the availability of technology, its advancements and this is being guided by laboratory/experimental findings to trace product quality. Moreover, this means the dissemination of NIRS methods should come to recognition irrespective of the region (i.e. whether developed or underdeveloped regions) to harness the full benefits the technology comes with.

Considering that the non-destructive technology also has shortfalls. For instance, NIRS calibration models to predict purity of powdered horticultural products and subsequent adulteration contents are limited to predicting the type of adulterant(s) you have trained the models with (Esteki et al., 2018). Moreover, for spectroscopy to perform with precision it requires accurate reference method for sample quantification measurements (Lohumi et al., 2015). This holds for all non-destructive methods i.e. Vis-NIRS, FT-IR, hyperspectral imaging and Raman spectroscopies, they need constant improvement of models. Their cost of implementation is high given the fact that to make sense of spectral data one requires additional software with different chemometrics to draw important features of products (Huang et al., 2008; Kamruzzaman et al., 2015a). On top of that, instruments and software need technical servicing and upgrading from time to time (Su et al., 2017). This also means personnel performing the analysis of complex spectra must be highly trained. The increasing demand for authentic products and/ food adulteration analyses alerts the requirement to reduce the costs of non-invasive technologies for effective monitoring in commercial applications (Zhang et al., 2018).

8. Conclusion

For the past decade, near-infrared spectroscopies appear to be the methods of excellence to detect the adulteration of a wide range of horticultural powdered commodities. Compared to time-consuming, manual laborious methods that require highly trained personnel and continuous usage of reagents, NIR spectroscopies mode of action is fast, rapid and accurate. These methods are reagent free, no sample preparation is needed after the model is developed. Non-destructive technologies vary in terms of how they are being operated. However, the principle in their application is similar as their power is centered on using molecular vibrations to search for invisible differences of food powders that the naked eye is limited to.

It should also be noted, throughout the improvement and progression of NIR techniques to monitor adulteration, the availability of these technologies is arguably outspread to the commercial industries, production lines, in harbors where imported food are initially inspected, etc., however, these techniques are restricted at the supermarket nor the household. This then justifies quite a several incidents of purchasing imitation and adulterated foods. The agro-food industry is one of many country's greatest assets and it requires constant new products development, improvements and investments for its growth. From the authors perspective, as the food production industries are engaging more in adopting the 4^{th} industrial revolution (4IR) technologies, comprehensive adulteration monitoring methods, NIRS in particular, need to be made accessible in every step of the food value chain (i.e. their utilisation and availability should be included even at the supermarket service tills), along with the models researchers have developed to evaluate food adulteration and quality issues. Perhaps this could play a significant role and contribution in mitigating adulteration problems and that's where consumers could be assured and satisfied that the food is authentic.

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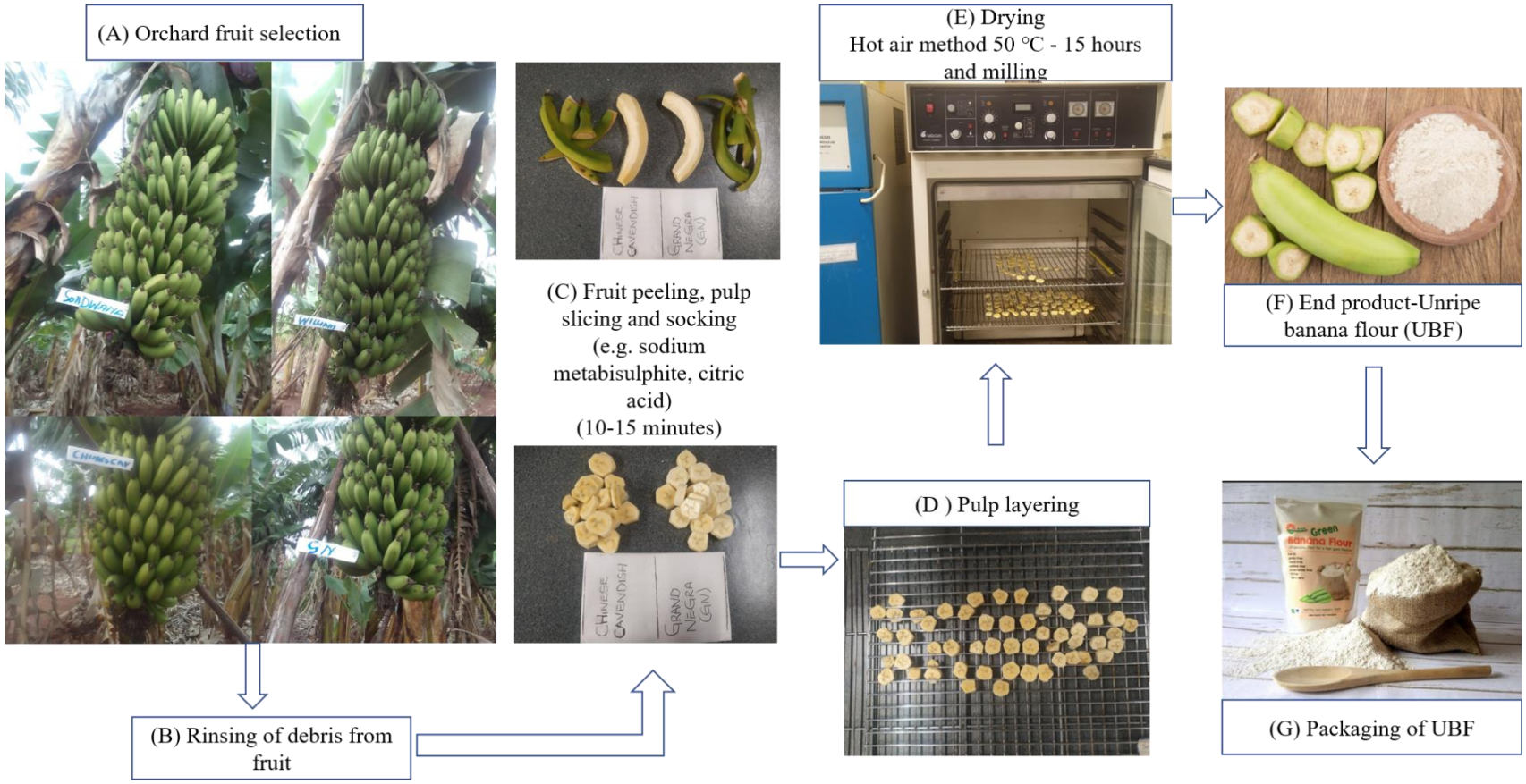

Figure 1: Process flow chart illustrating necessary steps to follow during the preparation of unripe banana flour. Source of (A): Original pictures by Mr Lucio Zuma and Mr John Mthethwa (Agricultural Research Council – Tropical and Subtropical Crops (ARC-TSC). Source of (F) and (G) M-Pak South African Food Review (2018). https://www.foodreview.co.za/green-banana-flour-for-a-healthy-lifestyle/ (Accessed 28 January 2019).

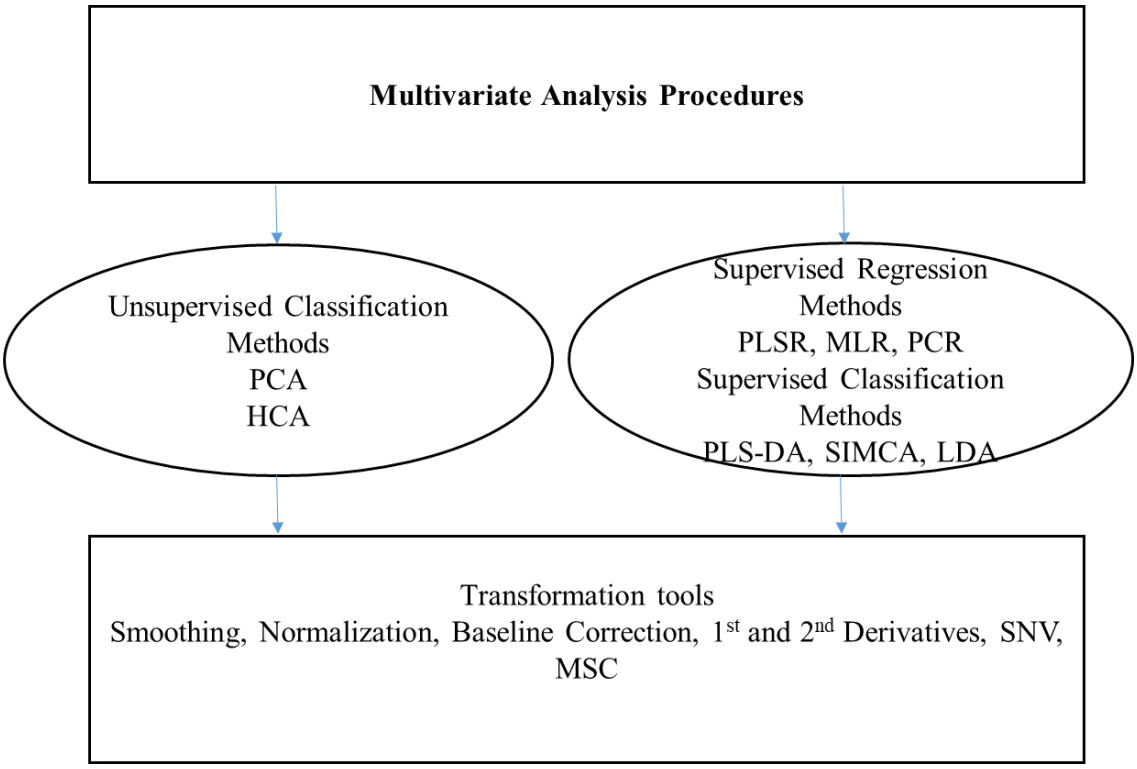

Figure 2: Frequently used chemometric methods to examine adulteration in powdered products. Adapted from (Pasquini, 2018).

Table 3: Recently published research on non-destructive techniques and chemometrics used to monitor adulteration of various categories of powdered horticultural products using.

Product type	Technique	Potential adulterant/s	Statistical Analysis	References
Spices and herbs	FT-IR			
Paprika powder		Suden dye, congo red dye	PLS-DA	Lohumi et al. (2017)
Paprika powder		Sudan I, Sudan IV, Lead (II, IV) oxide, Silicon dioxide, Polyvinly chloride, gum arabic	PCA, SIMCA	Horn et al. (2018)
Saffron		Turmeric, buddleja, gardenia, safflower, calendula	PCA, PLS-DA, siPLS	Petrakis and Polissiou (2017)
Oregano		Olive leaves, myrtle, sumac, cistus and hazelnut leaves	PCA, PLS-DA	Black et al. (2016)
Black pepper		Sorghum, Sichuan pepper, papaya seeds chili, black pepper husk, pinheads and defatted spent material	PCA GA-SVM, PLS-DA	Hu et al. (2018); Wilde et al. (2019)
Turmeric powder		Metalin yellow	PCR, PLSR	Dhakal et al. (2016)
Ceylon cinnamon	FT-NIR and FT-IR	Cassia cinnamon	PLSR	Yasmin et al. (2019)
Turmeric powder	FT-NIR	Corn starch	PCA, PLSR	Kar et al. (2019)
Chilli powder	Raman spectroscopy	Sudan dye I	PCA, PLS-DA	Haughey et al. (2015)
Paprika powder		Suden dye I	PCA, PLSR	Gao et al. (2015)

Table 3: Recently published research on non-destructive techniques and chemometrics used to monitor adulteration of various categories of powdered horticultural products using.

Product type	Technique	Potential adulterant/s	Statistical Analysis	References
Spices and herbs				
Turmeric powder	Vis-NIR spectroscopy	Metalin yellow	PCR, PLSR	Kar et al. (2018)
Paprika powder	NIR spectroscopy	Corn flour	LDA, PLSR	Zaukuu et al. (2019)
Chilli powder	UV-Vis spectroscopy	Suden III and Suden IV	ANN, PCR, PLSR	Ismal et al. (2018)
		Rhodamine B and red textile dye	PCA, PCA-DA	Rohaeti et al. (2018)
Black pepper powder	Hyperspectral imaging	Dried papaya seeds Buckwheat and millet	PCA, SIMCA PLSR	McGoverin et al. (2012) Orrillo et al. (2019)
Grounded red chilli		Salt, wheat flour, wheat bran and rice bran	SVM, PLSR	Khan et al. (2019)
Fruit and vegetable flour				
Onion powder	FT-IR	Corn starch	PLSR	Lohumi et al. (2014)
Cassava flour		Maleic acid	OCPLS, LS-SVM	Fu et al. (2017)
Garlic powder		Corn starch	PLSR	Lohumi et al. (2015)
Quinoa flour		Soybean, maize and wheat flours	SIMCA, PLS-DA	Rodriguez et al. (2019)

Table 3: Recently published research on non-destructive techniques and chemometrics used to monitor adulteration of various categories of powdered horticultural products using.

Product type	Technique	Potential adulterant/s	Statistical Analysis	References
Fruit and vegetable flour				
Purple sweet potato flour	Vis-NIR spectroscopy	White sweet potato flour	PCA RBF-PLS, KNN, LDA	Ding et al. (2015)
Hazelnut flour		Almond and chickpea flour	SIMCA	Lopez et al. (2014)
Unripe banana flour		Wheat flour	PCA, PLSR	Ndlovu et al. (2019)
Nutmeg powder	Hyperspectral imaging	Spent powder	PCA, ANN, PLS-DA	Kiani et al. (2019)
Hot beverage powders				
Grounded roasted coffee	Hyperspectral imaging	Roasted coffee husks, corn, barley, spent coffee	PLS-DA	Reis et al. (2017)
Cocoa powder	NIR	Carob flour	PCA, PLS-DA, PLSR	Quelal-Vásconez et al. (2018)
Cocoa powder	NIR	Cocoa shells	PCA, PLS-DA, PLSR	Quelal-Vásconez et al. (2019)
Green tea, green coffee	FT-IR	Sibutramine	PCA, HCA	Cebi et al. (2017)
LongJing tea	Raman spectroscopy	Lead chrome green	PLSR	Li et al. (2015)
Peaberry coffee (*Coffea canephora)*	UV-Vis-NIR	Normal coffee	PLS-DA, SIMCA	Suhandy and Yulia (2017)

Table 3: Recently published research on non-destructive techniques and chemometrics used to monitor adulteration of various categories of powdered horticultural products using.

Product type	**Technique**	**Potential adulterant/s**	**Statistical Analysis**	**References**
Medicinal powders				
Mixed herbal tea	FT-IR	Sibutramine	PCA, HCA	Cebi et al. (2017)
Notoginseng powder	Vis-NIR spectroscopy	*Sophora flavescens* powder and corn flour	PLS	Chen et al. (2019)
Lotus root powder	NIR spectroscopy	Cassava, sweet potato, potato and maize starches	PLSCM, SIMCA	Xu et al. (2013)
	FT-IR	Potato and sweet potato starches	PCA, PLS	Lui et al. (2013)
Gleditsia Sinensis thorn powder	FT-NIR spectroscopy	*Rosa multiflora* thumb and *Rosa rugose* thumb	PLSR, LDA, SVM, BPNN	Wang et al. (2018)

Table 4: Commonly used regression equations and statistical parameters measuring calibration and prediction accuracy of a PLS based model.

Description	**Equation expression**	**Reference**
Coefficient of determination	$R^2 = 1 - \frac{\Sigma(y_{cal} - y_{act})^2}{\Sigma(y_{cal} - y_{mean})^2}$	Williams (2003)
Root mean square error of calibration	$RMSEC = \sqrt{\Sigma(y_{cal} - y_{act})^2}/n$	Næs et al., (2004);
Root mean square error of cross validation	$RMSECV = \sqrt{\Sigma(y_{cal} - y_{act})^2}/n$	Nicolai (2007); Magwaza, et al., (2016)
Root mean square error of prediction	$RMSEP = \sqrt{\Sigma(y_{pred} - y_{act})^2}/n$	
Residual predictive deviation	$RPD = \frac{SD}{RMSEP}$	Davey et al. (2009); Bellon-Maurel et al. (2010)
Range error ratio	$RER = \frac{y_{max} - y_{min}}{RMSEP}$	Yasmin et al. (2019)

CHAPTER 3 - RAPID VISIBLE-NEAR INFRARED (VIS-NIR) SPECTROSCOPIC DETECTION AND QUANTIFICATION OF UNRIPE BANANA FLOUR ADULTERATION WITH WHEAT FLOUR

Abstract

Unripe banana flour is a premium nutritious product with a potential to curb degenerative diseases through resistant starch and gluten free traits, however, with scant techniques to monitor adulteration practices. The objective of the present study was to determine the efficacy of visible-near infrared spectroscopy (Vis-NIR) spectroscopy (Vis-NIRS) in the detection and quantification of unripe banana flour adulteration with wheat flour. Simulated adulteration of a composite banana flour was performed with different levels of wheat flour, in intervals of (2%) 20 g. kg^{-1}, ranging from 0-800 g. kg^{-1}. Each level was acquired in duplicate giving a total of 82 samples. Vis-NIR spectral data was acquired using a portable F-750 spectrometer in the range 447-1005 nm. Spectral data was analysed chemometrically using principle components analysis (PCA) and partial least squares regression (PLSR), with 41 samples used as a calibration set and 41 for validation. The first two principal components (PCs) accounted for 95% of spectral data variation, revealing five distinct clusters related to 0 g. kg^{-1}, 20-200 g. kg^{-1}, 220-400 g. kg^{-1}, 420-600 g. kg^{-1} and 620-800 g. kg^{-1} adulterated samples. The 2nd derivative Savisky-Golay (19-point smoothing, 2nd order polynomial) gave the best PLSR model, showing the highest R^2_c (0.991); R^2_p (0.993); RPD (12.021) and the lowest RMSEC (2.226 g. kg^{-1}) and RMSEP (1.993 g. kg^{-1}) values. In this dtudy, the developed Vis-NIRS PLSR models could therefore assist in controlling quality of unripe banana flour in the processing industries and in retail markets during product verification.

Keywords: Rapid detection, Unripe banana flour, Non-destructive technology, Chemometrics, Principal component analysis (PCA), Partial least squares regression (PLSR)

1. Introduction

Unripe banana flour (UBF) is one nutritious food product derived from a variety of *Musa* species (Singh et al. 2018), prepared by dehydrating fully matured green banana fruit pulp and milling them into powder (Agama-Acevedo et al. 2016). In various parts of Africa and international regions (Apostolopoulos et al. 2017), unripe banana flour is utilised as a staple food product and considered a substitute for cereal flours (Anyasi et al. 2013). Recently, commercial production and marketing of unripe banana flour has increased globally and this stems from its capacity to possess unique nutritional qualities (Farage et al. 2017). Banana flour is gluten free and contains high proportions of resistant starch (Patiño-Rodríguez et al. 2018). Daily consumption of unripe banana flour improves insulin sensitivity *in vitro* (Dan et al. 2015), stabilise blood glucose levels, promote gastrointestinal hormones and induces satiety, *in vivo* (Scarminio et al. 2012). Moreover, prebiotic properties of unripe banana flour have been reported to induce high production of short chain fatty acids, responsible for intestinal tissue protection (Almeida-Junior et al. 2017). Thus, health strategies to treat and combat illnesses such as colon cancer recommends the inclusion of unripe banana flour as food ingredient for gluten intolerant individuals (Torres et al. 2017).

In general, unripe banana flour by visual or colour inspection resembles similar physical properties as those of cereal flours, depending on the cultivar source (Kongolo et al. 2017). Wheat flour is among the inexpensive commercially traded staple cereal flours (Su et al. 2017). For these reasons,

and as a highly priced commodity, intentional and incidental banana flour adulteration practices with cheaper wheat flours, is thus anticipated and suspected to be a common practice (McGoverin et al. 2012). Adulteration is usually motivated by greed for increasing output and profit margins by the producers (Lohumi 2015). Economic food adulteration compromises the nutritional quality, threatens consumers' health and causes unnecessary product loss (Jha et al. 2015). Adulteration scandal of spices with lead oxide for colour enlightenment resulted in more than 60% individuals hospitalised in some parts of India and European countries (Everstine and Kennedy 2013). Over a million pounds of Asian honey were banned in the European markets due to an illegal antibiotic and artificial sweeteners found in honey (Schneider 2011). Through light microscopy, over 80% adulteration incidents in commercial unripe banana flour was reported in Brazil (Sardá et al. 2016). Findings by these authors revealed that flours commercially labelled as unripe banana flour products had starch granule structures matching those found in cereal flours and the majority of unripe banana flour samples evaluated were identified to have been prepared from ripe bananas with peels rather that unripe banana pulp. Therefore, to comply with food safety and quality control standards, it is of paramount importance to develop techniques that can rapidly detect and separate between the real and simulated unripe banana flour products.

In combination with chemometrics analysis, visible-near infrared spectroscopy (Vis-NIRS) technique serves as a reliable, rapid, non-destructive and considerably low cost method to investigate unauthorized food practices by simply constructing calibration and validation models (Qu et al. 2015). Chemometrics, usually applied to spectral data in the form of multivariate techniques, such as principal component analysis (PCA) and partial least squares regression

(PLSR) (Mahesh et al. 2015), provide interpretation to relevant invisible chemical information captured in the spectral region (Moscetti et al. 2015).

There has been no research on the use of the Vis-NIRS technique to detect adulteration of unripe banana flour with wheat flour. Therefore, the aim of the current study was to evaluate the potential of using a portable NIR spectrometer in association with multivariate analysis to develop prediction models for detecting unripe banana flour adulteration with wheat flour. The output obtained from this research could provide an approach for commercial mill factories to monitor process quality control of unripe banana flour, its derived products, and could facilitate product verification in retail markets.

2. Materials and methods

2.1. Preparation of unripe banana and wheat flours

Composite unripe banana flour was prepared from 23 fully matured (fingers at three quarters full stage) green bananas. The fruit were from different varieties of dessert and plantain cultivated at the Agricultural Research Council-Tropical and Subtropical Crops (ARC-TSC) farm in Burgershall Research Station, South Africa (25° 6′0″ S, 31° 4′60″ E). The fruit included 10 desserts (Chinese Cavendish, Dwarf Cavendish, Gros Michel, Grand Negra, Valery, Williams, and ARC-TSC breed selections: D11, MCC, PK6, and Sordwana) and 11 additional *Musa* genotypes (Calcutta 4, Ducasse, Fhia-01, Fhia-18, Foconnah, Hinoon, Green Red, Gold Finger, IPB5-61, Khai Thong, Lady Finger, Prata Anna, Pome) varieties. Green bananas were rinsed with tap water, carefully hand peeled to ensure complete skin removal. To prevent browning, the pulp was

immediately immersed in sodium metabisulphite solution (1.25 g. L^{-1}) for approximately 10-20 minutes. The pulp was then sliced using a vegetable cutting machine (HLC-300, Omcan, Niagara, NY) equipped with a P4 disc. The slices were dried at 50 °C for 15 hours using a commercial scale convective hot-air dryer (AD3000 Agri-Dryer, Dryers for Africa, Limestone Hill, SA). The relative humidity (RH) and air velocity were kept constant at 15±2% and 0.3 m. s^{-1}, respectively. Hot air-dried banana chips were ground in a laboratory milling machine (S8 Range, Drotsky Aktief (Pty) Ltd, SA) fitted with 0.8 mm sieve.

The obtained banana flour was immediately packed in high density polyethylene bags (300 mm x 450 mm), sealed and stored in boxes at room temperature till further use. To obtain the composite flour, about 100 grams, from each of the 23 prepared banana flour types, were combined to produce single composite banana flour. The banana flour was determined to contain ash (22.4 g. kg^{-1}), crude fibre (16.2 g. kg^{-1}), protein content (55.1 g. kg^{-1}) and had a moisture content of 9.5. The adulterant, wheat flour (SASKO, Pioneer Foods (Pty) Ltd, South Africa) contianing the following typical nutritional contents i.e. protein (102 g. kg^{-1}), carbohydrate (710 g. kg^{-1}), total fat (9 g. kg^{-1}) dietary fibre (37 g. kg^{-1}) ((AOAC 991.43) (nutritional information as packed)) was procured from the local market. Prior adulteration runs and spectra acquisition, unripe banana and wheat flours were further filtered (repeated 3 times) in a 355 microns sieve (Universal laboratory test sieve, SABS, SA) in order to ensure particle size homogeneity. Approximately 200 g. kg^{-1} composite unripe banana flour was weighed (Electronic Balance (BL-3200H), Shimadzu Corporation, Japan), and decanted into airtight plastic mixing container where subsequent adulterant levels of (0-800 g. kg^{-1}) wheat flour were added and thoroughly mixed.

2.2. Visible-Near infrared (Vis-NIR) spectra collection

Training set spectra were obtained using a portable Vis-NIR spectrometer (F-750 Produce Quality meter, Felix Instruments, Camas, WA 98607, USA) equipped with a Xenon Tungsten lamp, and recorded each spectral measurement in absorbance/and reflectance mode at 3 nm interval sampling. Approximately, 5 g (+/- 1 g) adulterated mixture was weighed (Electronic Balance (BL-3200H), Shimadzu Corporation, Japan) and transferred into petri dishes (50 mm x 55 mm), evenly distributed and enclosed in machine sample holder during scanning to prevent light escape. Each sample was scanned three times at three different controlled temperature environments (20, 25 and 30 °C), and a total of 82 spectra were collected per temperature. However, no significant variations were observed in developed calibration models at the afore-mentioned temperatures. Similar observations were reported by Ncama et al. (2018) using the same instrument. Thus, the results reported herein were on calibration models developed at 20 °C.

2.3. Chemometric analysis

Chemometric analysis was performed using the Unscrambler chemometric software (The Unscrambler X Version 10.3; Camo Process, SA, Trondheim, Norway). The margins of the acquired spectral range were curtailed to a range of 447-1005 nm to reduce noise in the dataset. For more precise prediction models, it is necessary to pre-process data in order to autocorrect for possible spectral shifts not relating to samples desired characteristics resulting from either light scatter or materials changing molecular response to light (Nicolai et al. 2007). Thus, in this study prior to PCA examination and PLSR modelling, pre-processing was done using Savitzky-Golay smoothing (Savitzky and Golay 1964), normalisation, Savitzky-Golay first derivative (7-point

smoothing, 2nd order polynomial), Savitzky-Golay second derivative (19-point smoothing, 2nd order polynomial), baseline correction and standard normal variate (SNV) (Rinnan et al. 2009). In each case, the resultant outputs were evaluated and juxtaposed. Both the number of principal components (7 PCs) used in PCA classification and the number of factors (7) for constructing PLSR models for the detection of unripe banana flour adulteration were from Unscrambler X software default settings.

2.3.1. Principal components analysis and outlier detection

PCA analysis, based on the adulterant percentage added into UBF, the spectra were split into 4 category variable groups, which consisted 10 spectral data sets each ranging from (20-800 g. kg^{-1}) 20-200 g. kg^{-1}, 220-400 g. kg^{-1}, 420-600 g. kg^{-1}, 620-800 g. kg^{-1}, excluding the pure/unadulterated UBF samples 0% (0 g. kg^{-1}). The data were mean centred, and then using random sampling cross validation the similarities and differences in samples chemical composition were assessed in PCA scores plot. Loadings plot was used to select, establish effective wavelengths, eliminate possible noise and spectral outliers. In order to identify outliers, which are samples far away from the zero line of the influence plot, Hoteling's T^2 with a 5% cut off set was implemented, and no outliers were diagnosed.

2.3.2. Partial least squares regression analysis

Partial least squares regression modelling was also constructed on mean-centred spectral dataset based on the test set validation method. The calibration set consisted of 41 specimen and 41 specimen were utilsed as the validation set. Using non-linear iterative partial least squares

(NIPALS) algorithm, spectroscopic absorbance intensities were converted to log absorbance values (log 1/R) and a linear correlation between the intensities and corresponding adulterating wheat concentrations was established. The performance of the resultant PLSR models was compared.

2.3.3. Determination of model accuracy

The optimal model performance was based on evaluating the calibration and validation sets using the following statistical parameters: coefficient of determination (R^2_c and R^2_p) (Eq. 1), root mean square error of calibration (RMSEC) (Eq. 2) and root mean square error validation/prediction (RMSEP) (Eq. 3) (Naes and Nyvold 2004). Bias, referred to as the average difference between predicted and reference values (Eq. 4) and the residual predictive deviation (RPD), considered as the dimensionless ratio of standard error of prediction to standard deviation of lab measured reference values as shown in (Eq. 5) (Davey et al. 2009) were included as statistical measures.

$$R^2 = 1 - \frac{\Sigma(ycal - yact)^2}{\Sigma(ycal - ymean)^2} \quad (1)$$

$$RMSEC = \sqrt{\Sigma(ycal - yact)^2}/n \quad (2)$$

$$RMSEP = \sqrt{\Sigma(ypred - yact)^2}/n \quad (3)$$

$$Bias = \frac{1}{n}\sqrt{\Sigma(ypred - yact)^2} \quad (4)$$

$$RPD = \frac{SD}{RMSEP} \quad (5)$$

where: y_{cal} is the calculated value, y_{act} is the actual measured value, y_{pred} is predicted value, y_{mean} is average value of predicted data, n is number of spectra and SD is the standard deviation of reference measured values.

3. Results and discussion

3.1. Vis-NIR spectra characterisation

Pure (UBF) and adulterated (UBF+ wheat flour) spectra before pre-processing are presented in Fig. 1 while Fig. 2 represent spectra which underwent second derivative (Savitzky-Golay-& 19-point smoothing, 2nd order polynomial) pre-treatment. As can be observed from the spectral patterns (Fig. 1 and Fig. 2), adulterated and pure UBF samples revealed similar spectra appearances and patterns except in some distinct wavelength throughout the spectral region studied (447-1005 nm). This suggested that spectra assignment bands for both pure UBF and with wheat flour contamination exhibit similar physical and chemical constituents. The application of 2^{nd} derivative transformation had a positive influence in making hidden spectral peaks visible (Fig. 1) in both pure and adulterated UBF samples (Fig. 2). This is clearly shown in Fig. 2 where differences in absorbance peaks across the studied region are revealed, possibly due to the variation in functional groups (C-H, O-H, N-H) in the sample mixture (UBF + wheat flour) resulting from the increase in the adulterant levels.

The most noticeable difference in wavelength bands that were useful in detecting the presence of wheat flour in UBF were obvious in the visible region (i.e. 479-483, 519, 573, 654 nm) and near infrared region (i.e. 717, 870; 897 and 951 nm) (Fig. 2). These absorption bands are a representative of specific functional groups describing physical, and chemical properties of various compounds found or picked up by the spectroscopy (Magwaza et al. 2016). Most prominently found absorption assignment peaks of Vis-NIR spectra of foods relate to overtones and combination bands of the fundamental molecular vibrations of C-H, O-H, N-H (Magwaza et al.

2016) and possibly C=O organic groups (Nawrocka and Lamorska 2013). Therefore, the separation between pure and adulterated UBF samples observed in the spectral region in this study (477-1005 nm) was based on matching the revealed spectral peaks with corresponding functional groups (related to chemical compounds) that absorbs in the Vis-NIR region.

From Fig. 2, a stretch/bending of wavelength pattern occurring from positive towards negative absorption assignment at 479-483 nm was revealed. In addition, a continuous shift in wavelength assignments from low absorption to high absorption state at 519 nm and wavebands at 573 and 654 nm region were observed. The absorption bands observed in the visible region (i.e. 479-483, 519, 573, 654 nm) of the study were associated to the transition of chromophores (Mishra et al. 2015), which are molecules that give agricultural food matrices colour/pigmentation and that strongly absorb light in the visible region (400-700 nm) of the electromagnetic spectrum (Ambrose and Cho 2014). Chromophores in powdered food materials involve a series of conjugated bonds and aromatic ring compounds containing C=O and C=C organic molecules (Mishra et al. 2015). Therefore, in the current research, the observed wavelength bands separating the adulterated UBF samples with pure UBF samples at 479-483 nm; 519, 573 and 654 nm (Fig. 2) could then be suggested to have resulted from a sequence of conjugated double bonds of C=O and or, C=C associated with different colour compounds formulation resulting from interaction of wheat flour and UBF samples. The absorption peaks at 717 nm were related to combination of C-S stretching, while wavebands at 870; 897 and 951 nm were ascribed to second overtone of N-H and O-H; and third overtone of C-H and N-H stretching organic compounds as suggested by (Stuart 2004; Osborne 2006).

3.2. Chemometric analysis

A combined explained variance of > 90% for the first and second principal components (PCs) was observed for all the pre-processing techniques. The principal component analysis (PCA) scores plot in Fig. 3 depicts first derivative corrected spectra classification patterns between the 0% (0 g. kg^{-1} or no wheat flour added) and adulterated 2-20% (20-200 g. kg^{-1}), 22-40% (220-400 g. kg^{-1}), 44-60% (420-600 g. kg^{-1}) and 62-80% (620-800 g. kg^{-1}) UBF samples. The first two principal components yielded 95% explained variation in sample data set, with PC1 having 92% variation while PC2 had 3% having PC1 = 92% and PC2 = 3%.

Table 1 provides a summary of PLSR modelling results obtained using raw spectra and different spectral pre-processing techniques along with statistical parameters used for determining models accuracy in predicting wheat in UBF samples. The performance of PLS regression models and optimum pre-processing method to describe the variation between spectral (X) variables and adulterants concentration (Y) was selected based on higher R^2_p and RPD; i.e. with R^2_p values approaching one and RPD greater than three and lower RMSEP (Bellon-Maurel et al. 2010). All models developed to predict wheat flour in UBF were successful and showed good stable coefficient of determination for calibration and prediction sets (R^2_c and R^2_p) above 0.98.

The generated regression models revealed that models performance created using the raw spectra were similar to those pre-treated with S-Golay Smoothing technique, however, pre-processing improved the resultant models (Table 1). PLS regression developed using second derivative (S-Golay 19-point smoothing, 2^{nd} order polynomial) showed R^2_c (0.991), RMSEC (2.226 g. kg^{-1}), R^2_p (0.993) and RMSEP (1.993 g. kg^{-1}) in the calibration and validation models, respectively.

Furthermore, constructed prediction models were verified for reliability by calculating their RPD values. The RPD values of 1-1.5 infer unreliable models; values between ≥ 1.5-2.5 are considered fair for rough predictions in screening and breeding programs; RPD values ≥ 3 are potentially regarded as satisfactory and useful in monitoring quality in food analysis systems, according to (Bellon-Maurel 2010). In this study, the RPD values for determining models' reliability were above 3 throughout the PLSR modelling results obtained (Table 1). This inferred that our generated PLSR models could be beneficial during quality control in the detection of unripe banana flour adulteration with wheat flour.

The best PLSR model with optimal pre-processing method selected based on higher R^2_p and lower RMSEP and high RPD value was the second derivative (Savitzky-Golay, 19-point smoothing, 2nd order polynomial). This model showed the best capabilities to detect adulteration of banana flour with wheat flour (Table 1), indicating the power of pre-treating spectra before modelling improves the quality and robustness of the prediction models. PLSR prediction plots confirming the linear mathematical relationships between spectral variation (X) and corresponding actual measured wheat flour values (Y) for calibration (training) and validation/test sets are depicted in Fig. 4.

4. Conclusion

The detection of wheat flour adulteration contents in unripe banana flour using Vis-NIR spectroscopy coupled with chemometrics tools were performed successfully. PCA applied to Vis-NIRS data showed successful pattern visualisation between pure and adulterated banana flour samples with the use of Savisky-Golay derivative pre-treatment. PLS regression showed excellent prediction models with similar accuracy throughout used pre-processing methods with second

derivative Savisky-Golay having the highest prediction precision R^2_p (0.993); RPD value of 12.021 and lowest RMSEP = (1.993 g. kg^{-1}) values. This is the first report on the application of Vis-NIR spectroscopy to detect adulteration of unripe banana flour with a cereal flour. The results from the present study will facilitate development of methods for rapid detection of banana flour adulteration with wheat flours.

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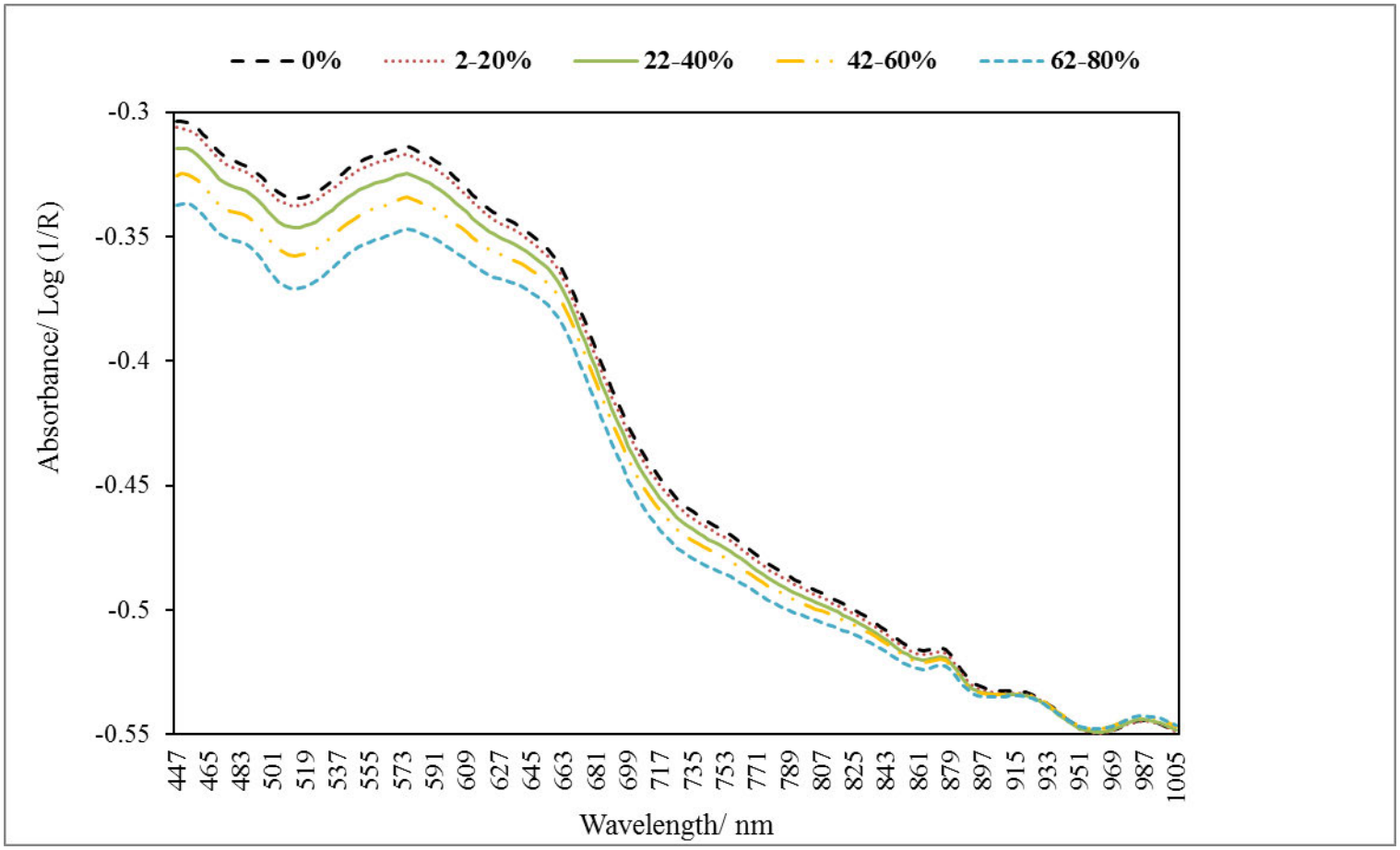

Figure 1: Typical unripe banana flour reduced-average absorbance spectra Log (1/R) with different levels of adulteration before pre-treatment.

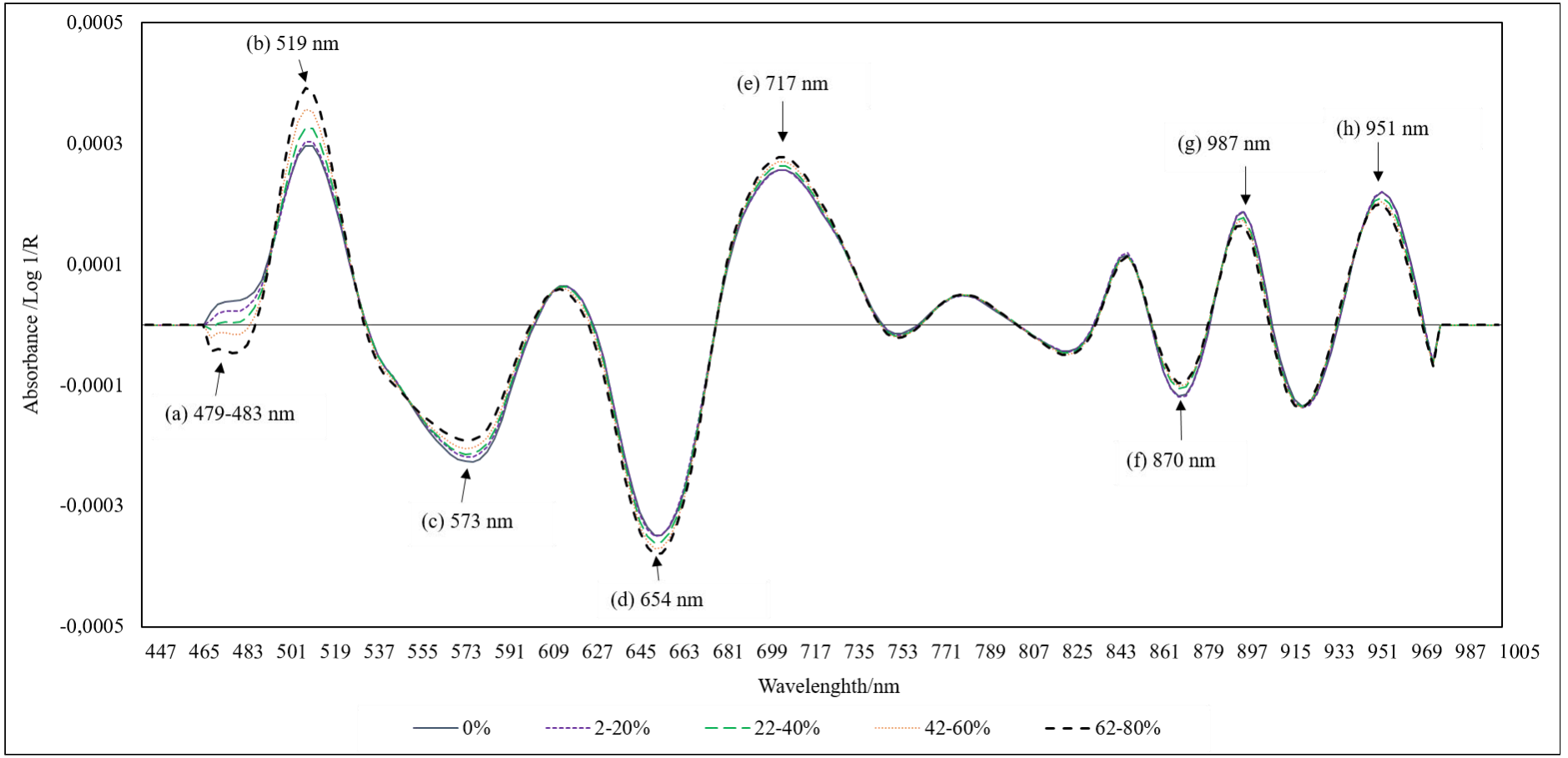

Figure 2: Reduced to average spectra of UBF (0%) with different mixes of wheat flour adulterant (2-20%; 22-40%; 42-60% & 62-80%) after 2nd derivative Savisky-Golay (19- point smoothing, 2nd order polynomial).

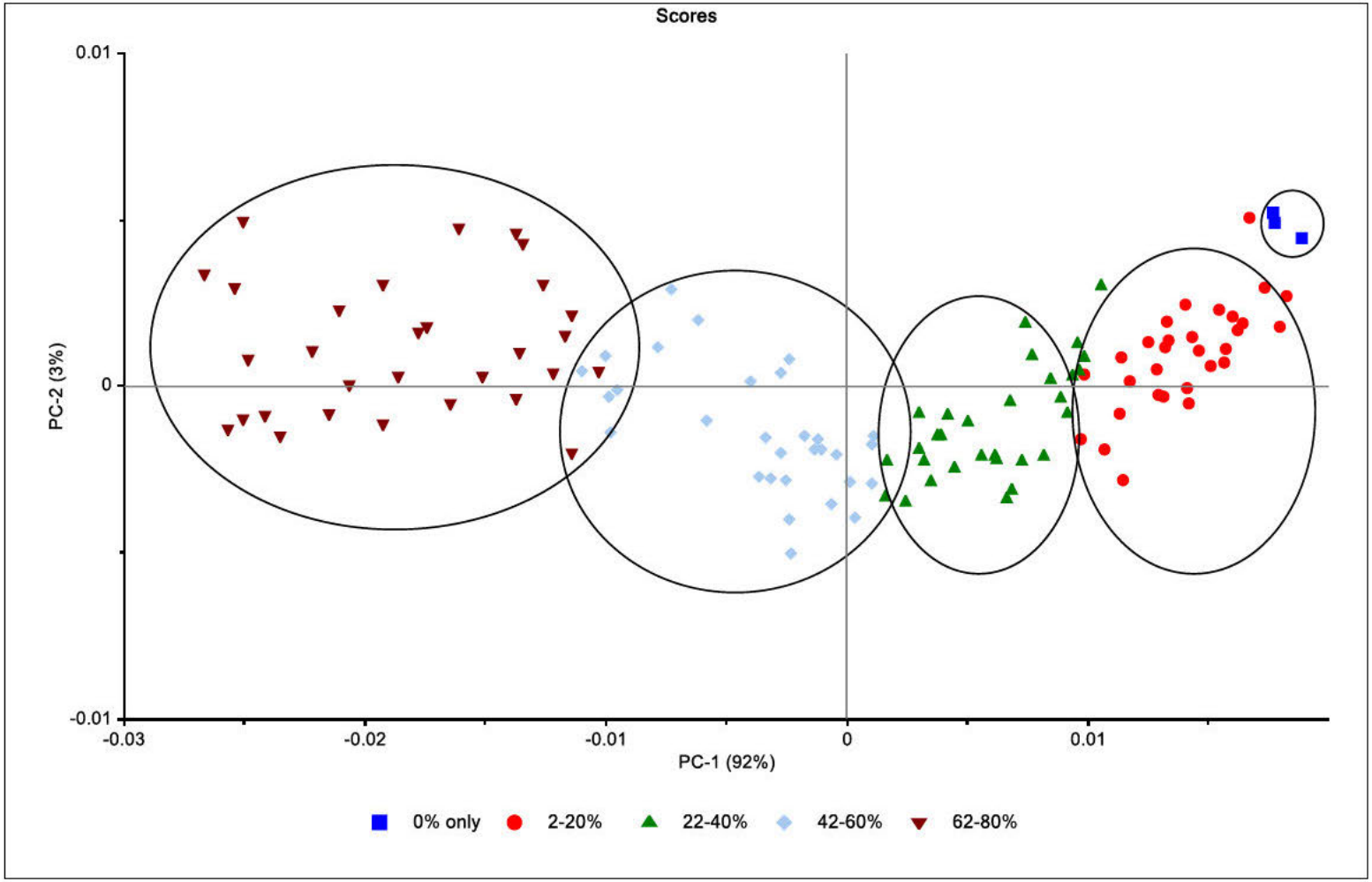

Figure 3: PCA scores plot (PC1-PC2) classifying pure and wheat flour adulterated (2-80%) UBF samples after 1st derivative Savisky-Golay (7-point smoothing, 2nd order polynomial).

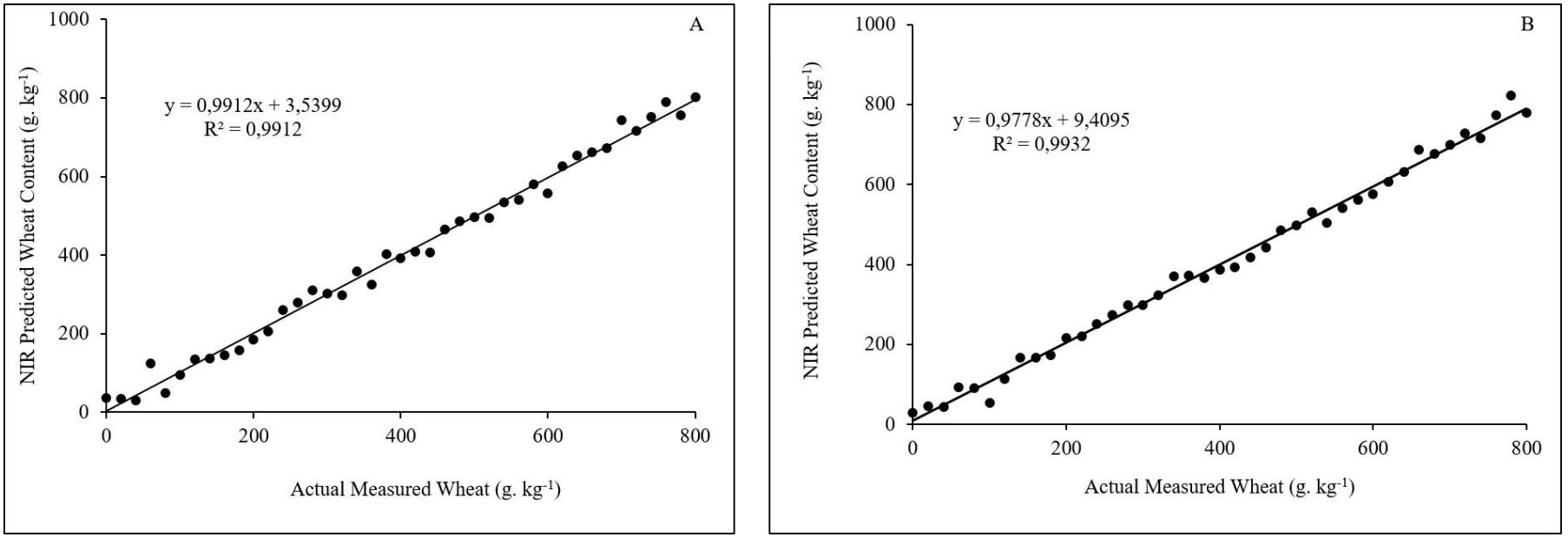

Figure 4: PLS calibration (A) and validation (B) scatter plots showing linear relationship between NIR predicted against corresponding added wheat flour percentages (20-800 g. kg^{-1}) taken after 2^{nd} derivative (S-Golay, 19- point smoothing, 2^{nd} order polynomial).

Table 1: Performance of PLSR models in predicting the amount of adulterant (wheat flour) in unripe banana flour using absorbance spectra and different pre-treatment methods.

No. of Factors	Pre-processing method	Training set		Statistical Validation	Parameters set		
		R^2_c	RMSEC	R^2_p	RMSEP	Bias	RPD
3	Raw spectra	0,987	2,709	0,987	2,737	0,023	8.753
3	S-G Smoothing	0,987	2,718	0,987	2,741	0,025	8.740
2	Normalisation	0,985	2,868	0,983	3,063	-0,156	7.822
2	1st Deriv. S-G (7 Point)	0,989	2,375	0,991	2,189	-0,129	10.945
2	2nd Deriv. S-G (19 Point)	0,991	2,226	0,993	1,993	0,052	12,021
2	Baseline	0,989	2,437	0,988	2,6075	0,187	9,188
1	SNV	0,978	3,505	0,979	3,404	-0,081	7,038

R^2_c: coefficient of determination for calibration; R^2_p: coefficient of determination for validation; RMSEC: root mean square error of calibration; RMSEP: root mean square error of prediction; RPD: residual predictive deviation; S-G: Savitzky-Golay; 1st Deriv.: first derivative; 2nd Deriv.: second derivative; SNV: Standard Normal Variate.

CHAPTER 4 - DEVELOPMENT OF A NON-DESTRUCTIVE PARTIAL LEAST SQUARES REGRESSION (PLSR) MODEL TO ASSESS UNRIPE BANANA FLOUR ADULTERATION WITH MAIZE (*ZEA MAYS*) FLOUR USING VISIBLE-NEAR INFRARED SPECTROSCOPY

Abstract

Rapid detection of processed products' economic adulteration is necessary since the presence of various adulterants is very likely to reduce the functional ingredient potency, and in worst cases, impacts negatively on the health of consumers. A portable visible-near infrared (Vis-NIR) quality meter was utilised as a tool to detect maize flour adulteration in unripe banana flour (UBF). To quantify UBF adulteration with maize flour, the relationship between UBF spectra and the adulterant were examined by partial least squares regression using Kernel algorithm through selected wavelengths (full wavelength region 447-1020 nm, visible region 447-702 nm and near infrared region 705-1020 nm) and pre-processing methods (MSC and 1st derivative Savitsky-Golay, 2nd order polynomial). A total of 126 data samples were collected for training (n = 84) and validation (n = 42) of PLSR models with an additional 126 data samples gathered for external testing the models. Coefficient of determination for validation models (R^2_v) ranged from 0.949 to 0.961 at 447-1020 nm, 0.813 to 0.834 at 447-702 nm and 0.902 to 0.906 at 705-1020 nm wavelength. Prediction models based on the external test set samples demonstrated that the developed PLS regression models could be confidently utilised for the prediction of UBF adulteration with maize flour despite change in wavelength and temperature surroundings; giving significant R^2_p, ranging from 0.904 to 0.922 (447-1020 nm), 0.694 to 0.831 (477- 702 nm), 0.534 to 0.761 (705-1020 nm). RPD values ranged from 3.239 to 3.603 at 447-1020 nm; 1.816 to 2.445 at 447-702 nm and 1.471 to 2.052 at 705-1020 nm.

Good prediction models developed in this study with full Vis-NIR region demonstrated the suitability of a handheld visible-near infrared (Vis-NIR) spectroscopy as a non-destructive, robust and environmental friendly measure to monitor quality and screen for possible adulteration by banana flour producers in pack houses and processing units.

Keywords: F-750 NIR spectroscopy, chemometric analysis, effective wavelength, maize flour quantification, banana flour authentication

1. Introduction

The rise in the consumption of unripe banana flour (UBF) in recent years has been driven by its acknowledgement as a natural gluten-free alternative for the ordinary staple flours (Singh et al., 2016). Research findings in various literature revealed that multiple utilisation of unripe banana flour depends on consumers' changing lifestyle patterns and preferences which are in turn driven by growing awareness concerning resistant starch functionality (Zandonadi et al., 2012; De Gouveia et al., 2013). Banana flour applications in the food industry have demonstrated to have a significant potential during the development of gluten-free snacks (Agama-Acevedo et al., 2009), pasta (Flores-Silva et al., 2015; Almanza-Benitez, et al., 2015), weaning products, various bakery and beverage products (Anyasi et al., 2013; Sarawong et al., 2014).

In developing countries such as South Africa, banana flour has been endorsed by organizations like Diabetes SA to control blood glucose and insulin level for diabetic patients (Future Market Insight, 2018). However, the concern with banana flour industry is that currently there are no specific quality and safety monitoring system in place for the major producing countries which

include Canada, Brazil, China, Australia, India, United States, including Africa and Southern African countries (Sardá et al., 2016a). Most recent research studies on banana flour have focused on physico-chemical properties (Savlak et al., 2016), *in vitro* and *in vivo* studies for reducing the risk of non-communicable diseases (Sardá et al., 2016b; Bi et al., 2017), its utilization to create edible and biodegradable films (Gutiérrez et al, 2016) to mention a few.

Nowadays, consumers are becoming skeptical about the quality, authenticity, the manner in which food products are processed, chemical and nutritional composition as well as the safeguard with respect to microbial, toxic and inferior contamination of edible agricultural products (Borràs et al., 2015). In the fore-mentioned countries involved in the production of banana flour, to our knowledge no studies have been reported regarding assessing the products' adulteration incidents despite valuable health benefits it has on human physiology and is considered a replacement for stable flours. With this gap of information, in a niche market, opportunities for the products' exposure to unfair trade involving contamination due to negligence may be introduced. Moreover, economic adulteration, which is a widespread issue in the processing industry of powdered food materials accompanied with false product labelling is also motivated by unfair producers.

Food products most likely targets for adulteration practices include those in demand by the populace, of high-value and which go through strict processing protocols before marketing (Manning et al., 2016; Hong et al., 2017). Strictly, unripe banana flour is produced from matured green banana fruit pulp and processed before any ripening initiation within the first 48 hours' post-harvest (Menezes et al., 2011). Issues of authentication and adulteration in the commercial unripe banana flour supply chain have been documented, coincidently, with greater than seventy percent incidents in developed countries' such as Brazil for the year 2016. It is

worthy to note that adulteration with respect to unripe banana flour comes in different forms *viz*, flour processed from banana fruit pulp in an advanced stage of ripening, UBF prepared from pulp with peel traces, and banana flour which has undergone entire substitution or mixing with staple flours (Sardá et al., 2016a). With the latter, considering the sensory nature (*i.e.* colour, texture and particle size) of cereal flours, such as maize (*Zea mays)* flour, makes adulteration of banana flour possible without the consumer noticing.

From the nutrition perspective, dilution or complete substitution of unripe banana flour with commonly known flours deteriorates the premium quality of the product. In addition, in the food operating systems, engagement of any sort of fraud interrupts product distribution chains (Bogadi et al., 2016), mislead various diet and raises health problems as certain consumers may be allergic to the adulterant material as mentioned in various studies in the literature (Spink et al., 2011; Fu et al., 2014; Xu et al., 2015). Thus, in order to prevent the occurrence of unripe banana flour adulteration in the national and international market, the industry seeks and requires a rapid and sensitive economic tool to enable banana flour protection against sophisticated adulteration acts.

The authentication of unripe banana flour is crucial for public health and the industry, hence the requirement of techniques to monitor its processing involving adulteration practices. In this regard, visible-near infrared (Vis-NIR) spectroscopy, is one of the well-researched and approved vibrational method deemed advanced to cater for adulteration problems which has not yet been highly applied to studies pertaining banana flour. The robustness of the technique made understandable with multivariate analysis partial least squares regression (PLSR) have identified contamination of milk with melamine (Fu et al., 2014; Domingo et al., 2014) purple sweet potato flour mixed with white sweet potato (Ding et al., 2015) to mention a few. As

recommended by many authors PLSR is the most suitable statistical analysis for dealing with successive near-infrared spectra and building spectroscopic calibration models (Balabin and Lomakina, 2011) as it can relate spectral training data matrix (*X*) to the reference measured variables of analyte (*Y*) (Godoy, et al., 2014). The objective of the current study was, therefore undertaken to investigate the applicability of portable Vis-NIR spectroscopy to detect adulteration of unripe banana flour with maize (*Zea mays*) flour. In doing this, partial least squares regression (PLSR) was employed to construct detection models to differentiate UBF adulteration mixes with maize flour. The developed monitoring and quality evaluation models based on infrared technology (Vis-NIRS) are anticipated to provide banana flour producers with a fast strategy to assess the authenticity of the product. It is believed that the result obtained from this research would optimize the guaranteed quality of the product, increase consumers' confidence during purchasing of banana flour, hence, harnessing economic growth for producing countries.

2. Materials and methods

2.1. Sample preparation: unripe banana and maize flours

Unripe banana flour samples were prepared from 23 cultivars of fully matured green banana fruit at stage one according to the standards banana colour chat by SH Pratt & Co (Bananas) Ltd. (Luton) (Tapre and Jain, 2012). The fruit comprised dessert and plantain varieties cultivated at the Agricultural Research Council-Tropical and Subtropical Crops (ARC-TSC) farm in Burgershall Research Station, South Africa (25° 6′0″ S, 31° 4′60″ E). The fruit included 10 desserts (Chinesse Cavendish (AAA), Dwarf Cavendish (AAA), Grand Negra (AAA), Gros Michel (AAA), Valery (AAA, Williams (AAA),), and ARC-TSC breed selections: Sordwan (AAA), PK6 (AAAB), MCC (AAA), D11 (AAA)) and 13 additional *Musa*-genotypes (Calcutta

(AAA), IPB5-61 (AAA), Green Red (AAA), Pome (AAB), Lady Finger (AAB), Hinoon (AAAB), Fhia-18 (AAAB), Fhia-1 (AAAB), Gold Finger (AAB), Prata Anna (AAB), Ducasse (ABB), Khuai Thong Raung (AA), Foconnah (ABB) varieties.

Concisely, unripe banana flour samples were prepared by first peeling and uniformly slicing green banana pulp (HLC-300, Omcan, Niagara, NY). Prior dehydration, slices were immersed in sodium metabisulphite solution (0.0125 kg/L) for approximately 10-20 minutes, to prevent enzymatic browning. Dehyhdration of samples was performed utilising a convective hot-air dryer (AD3000 Agri-Dryer, Dryers for Africa, Limestone Hill, SA) at 50 ºC for a maximum duration of 15 hours. The conditions inside the dryer relating to relative humidity (RH) and air speed were maintained constant at 15±2% and 0.3 m/s, respectively, throughout the drying period. Taking equal weight (0.1 kg) each 23 prepared banana flour samples were miscellenoulsy combined in one bowl, with an aim to create a standard unripe banana flour which was then used in subsequent adulteration experiments.

To prepare maize flour (MF), IWISA samp (i.e. roughly or coarse grounded dried white maize seeds) were procured from a local supermarket and milled using a laboratory machine (S8 Range, Drotsky Aktief (Pty) Ltd, SA). Prior adulteration experiments and spectra acquisition, unripe banana and maize flours were further filtered (repeated thrice) in a 355 microns sieve (Universal laboratory test sieve, SABS, SA) in order to ensure particle size uniformity. Approximately 200 g/kg composite unripe banana flour was weighed (Electronic Balance (BL-3200H), Shimadzu Corporation, Japan), and decanted into airtight plastic mixing container where subsequent adulterant levels of (20-800 g/kg) maize flour were added and thoroughly mixed.

2.2. Vis-NIR data acquisition

The absorbance spectra of pure unripe banana and pure maize flours; and the adulterated UBF samples were recorded over a wavelength range of 285-1200 nm as programmed by a portable Vis-NIR spectrometer (F-750 Produce Quality meter, Felix Instruments, Camas, WA 98607, USA) at 20 ℃ controlled environment. The spectrophotometer was equipped with a Xenon Tungsten lamp and recorded each spectral measurement in absorbance mode at 3 nm interval sampling. Prior adulteration experiments, n = 3 scans of pure UBF and n = 3 pure samples from maize flour were collected, following the mixtures of UBF samples adulterated with maize flour. All measurements were prepared in triplicate in the range of 2-80% (20-800 g/kg) in increments of 2% resulting in a subtotal of 126 successive scans. The visible to near-infrared spectra acquired simultaneously (447-102 nm) were used in subsequent PLSR chemometrics analyses.

2.3. Spectra pre-processing and PLS modelling

Chemometrics analysis computations were implemented using the Unscrambler software (The Unscrambler X Version 10.3; Camo Process, SA, Trondheim, Norway). The beginning and ends of raw spectra gathered from the Vis-NIR spectrometer range (285-1200 nm) were characterised by noise and background shift in addition to samples important information. Hence, the regions at 285-444 nm and 1023-1200 nm were removed prior calibration and a new Vis-NIR range (447-1020 nm) was established and further divided into visible (447-702 nm) and NIR (705-1020 nm) wavelength regions. These wavelength segments were independently subjected to different pre-treatment tools in order to select the ideal wavelength region for best PLSR models.

Data modelling using selected chemometrics transformation techniques *viz*, 1st derivative Savitsky-Golay (2nd order polynomial), and multiplicative scatter correlation (MSC) were studied in order to reduce the dimensionality of the sample spectra data, to extract useful information from the complex spectra, and to obtain reliable accurate and stable models. To construct the PLS models spectral data were grouped into a 2:1 ratio. Using Kernel algorithm PLSR models were developed based on test set validation method. The test set validation method can be regarded as a dual approach of constructing the calibration model and testing for its stability by interchanging calibration data set and validation data samples during model development whilst observing that the obtained differences in the regression statistics are minimal. Randomly selected, 84 dataset samples were considered for building calibration models and 42 samples were used as a separate set for validating the models. Outliers were assessed by Hoteling's T^2 at 5% and F-residual, and no outliers were detected.

To test for models robustness and stability for future applications, it was thought necessary to examine the models using a new set of data samples obtained from a different experiment and environmental temperature settings. The external data set comprise a group of 126 samples (0-800 g/kg at 2% interval of adulterant dilution) each collected from independent experiments at temperature settings of 25 °C.

2.4. Evaluation of PLSR models

Statistical indexes such as coefficients of determination for calibration, validation and prediction (R^2_c, and R^2_p), root mean square errors for calibration, validation and prediction (RMSEC and RMSEP), the bias which refers to as the average difference between predicted and reference values as well as the ratio of performance to deviation (RPD) were evaluated. RPD measure the reliability of the PLSR models and is calculated by diving the standard

deviation of the measured adulteration concentration for the prediction set by RMSEP. As reported by Delin et al. (2012) when the RPD values are below 1.5 it means the calibration model is unusable, if the RPD values are between 2 and 2.5 then the calibration model is feasible, for values between 2.5 and 3.0 and above 3.0 it means the prediction accuracy of the model is excellent. The quality of the model for the study was evaluated by comparing each pre-processing method applied and selected based on lower RMSEs, higher R^2 and higher RPD values.

3. Results and discussion

3.1. Interpretation of spectral features for pure and adulterated samples

Near-infrared spectroscopies' ability to identify and discriminate between materials is based on the vibrational responses of chemical bonds to Vis-NIR radiation (Cozzolino and Murray, 2004). Moreover, Vis-NIR spectrometer intensities are better enhanced when utilised in combination with chemometric statistical tools and preprocessing techniques (Pomerantsev and Rodionova, 2012). Derivatives are mathematical transformation known to result in better illustrations of spectral data highlighting absorptions peaks with useful information that allows samples to be discriminated apart (Alishahi et al., 2010). Shown in Figure 1 are average 1^{st} derivative Savitsky-Golay log (1/R) (2^{nd} order polynomial) original spectra of pure UBF, pure MF and MF adulterated UBF samples for the entire visible-near infrared range. Comparable bands between pure spectra of UBF and MF samples could be observed in the Vis-NIR regions, however, with different intensities and this could be attributed to differences in the physical and chemical matrixes amongst the two products. From Figure 1, it can be observed that the absorption bands for pure MF are higher than that of pure UBF as well as the adulterated spectra (20-400 g/kg and 420-800 g/kg). The absorbance spectra for different concentrations of

adulterant in Figure 1 reveal that with the increase in the dose of adulterant there was also an increase in absorbance. From this observation it can then be suggested that PLSR 1st derivative Savitsky-Golay (7-point smoothing, 2nd order polynomial) could be used as an identification tool to discriminate pure UBF samples from those adulterated with maize flour.

In general, well-defined signatures with remarkable absorption bands were observed throughout the electromagnetic spectrum (447-1020 nm). As reported in various literature, light absorbance by infrared spectroscopy in the NIR (labelled as region B on Figure 1) associates with overtones and combination bands of broad overlapping fundamental vibrations of hydrogen bonds such as C-H, N-H and O-H (relating to carbohydrates, proteins and moisture; respectively) (Riedl et al., 2015) whilst the visible absorption bands (region A in Figure 1) involve the presence of different chromophores containing conjugated double bonds (C=C; C=O) (Zandomeneghi et al., 2000). Differences revealed in region A and B (visible and near infrared regions) were attributed to be due to changes in concentrations of principal functional groups relating to C=O, C=C, O-H, N-H and C-H, overtones and combination vibrations. The observed spectral peaks were related to pigmentation, moisture, proteins and carbohydrates structures; and these were implied to have been the important determinants of maize flour adulteration level in UBF samples.

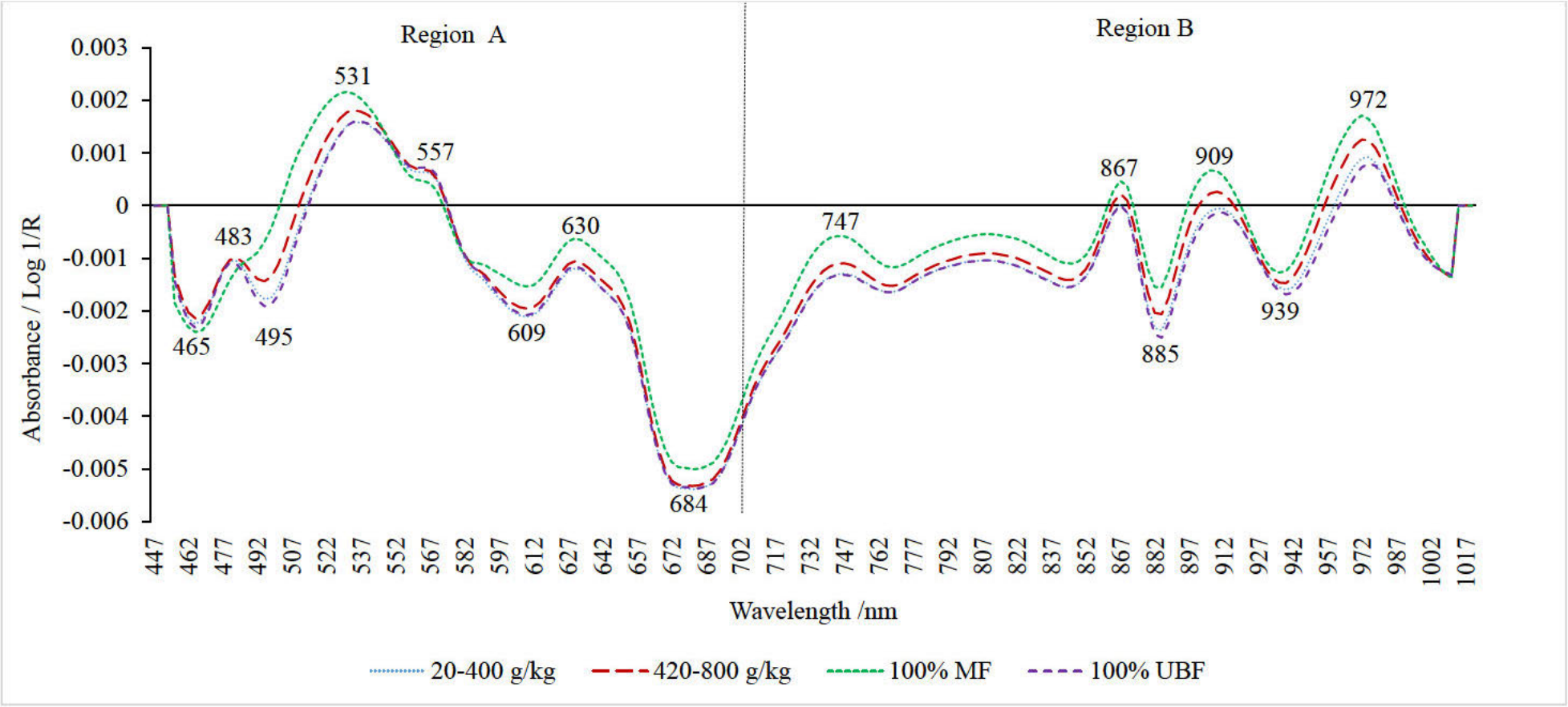

Figure 1: Mean absorbance spectra of samples in different adulteration group levels. (where: 100% UBF = pure unripe banana flour; 100% MF = pure maize flour; 20-400 g/kg and 420-800 g/kg = concentration ranges of MF in UBF).

3.2. PLSR calibration and prediction models

Partial least squares regression (PLSR) analysis is one of the commonly used methods applied in the quantification of adulteration contents in various food products (Kasemsumran et al., 2005). Multivariate calibration analysis performed by PLSR allows observations of linear mathematical correlation between independent variables *X* (spectral data) and dependent variable *Y* (concentration of adulterant) (Godoy et al., 2014). During the PLSR examination spectral dataset are compressed into orthogonal structures called latent variables (LV) or factors, and these are used to describe the maximum covariance between X (spectra) and Y (level of adulterant) variables (Vadivel et al., 2018). Various mathematical pre-treatment methods (i.e. MSC,1st derivative Savitsky-Golay, 7-point smoothing, 2nd order polynomial) were examined prior the development PLSR calibration models to discriminate pure UBF samples from different percentages of adulterant (maize flour) added based on the level of adulteration (20-800 g/kg). PLSR models were developed using the full wavelength range (447-1020 nm), the visible range (447-702 nm) and NIR segment (705-1020 nm) of the electromagnetic spectrum.

Depicted in Table 1 are PLSR models obtained from the study. In the current study, the application of PLSR with and without preprocessing method generated the most stable and accurate regression models from calibration to test set based on the evaluated full wavelength (447-1020 nm), visible (447-702 nm) and NIR (705-1020 nm) regions. To the best of our knowledge, this is the first research reporting on the non-destructive prediction of maize flour adulteration in unripe banana flour with NIR handheld spectroscopy. Thus, close observations were made with respect to temperature range (20 and 25 °C) and wavelength selection range. In this study, all calibration models were constructed and validated with separate samples obtained from an environmental setting of 20 °C. Thereafter the obtained models were tested

for future stability with spectra obtained on a separated set experiment at 25 °C. This was done to mimic possible future commercial applications of the handheld Vis-NIR spectrophotometer at a different ambient temperature environment. The external test set models reveal that the performance of the developed calibration and prediction models will remain satisfactory despite the change in temperature and wavelength range, provided un-preprocessed full Vis-NIR wavelength region is used (Table 1). As illustrated in Table 1, models obtained in the range of 447-1020 nm showed higher prediction R^2 of 90-96% accuracy and excellent RPD values ranging at 3.23-3.60 indicating the models are fit for quantitative prediction of maize flour adulteration in unripe banana flour. Whilst, the tested pre-treatment methods showed low predictive ability especialy in the range of 447-702 nm (1^{st} Der. S-Golay, (0.694)) and 705-1020 nm for both MSC (0.679) and 1^{st} Der.S-Golay (0.534), respectively. Even though this was the case, their RPD values (Table 1) indicated that these models were still appropriate for rough predictions as suggested by Saeys et al. (2005) and Zimmermann et al. (2007).

Plots showing actual versus the predicted concentration of maize flour in unripe banana flour from the PLS regression model for both temperature modes (20 and 25 °C) are displayed in Figure 2 below. Where Figure 2(A) demonstrates the validation plot model (at 20 °C) and Figure 2(B) is for external test set model (at 25 °C). In overall, the PLSR calibration models generated in this study have versatile applications and revealed adequate accuracy and stability.

On the authors perspective, calibration and prediction models with more accuracy fit were obtained with full Vis-NIR spectra (447-1020 nm) using raw spectra. As shown in Table 1 the R^2 for the full range was 0.955 for calibration, 0.961 for validation and 0.922 for external test prediction (Table 1). Based on the results of R^2 accuracy, RMSEP and RPD, models for un-preprocessed spectra gave better predictions, small RMSEP (4.801 (validation set), 6.734

(external tests set)) and high RPD (5.094 (validation data), 3.603 (test set data)) values. The authors then would recommend manufacture to consider using models for a full wavelength range for better quality and monitoring control during unripe banana flour adulteration detection with maize flour in future.

Table 1: Statistical parameters of PLSR models for prediction of maize flour in unripe banana flour based on selected wavelength regions and different transformation methods.

			Calibration (20 °C)		Validation (20 °C)				Test (25 °C)			
Pre-treatment	π (nm)	LV	R^2_c	RMSEC	R^2_v	RMSEP	Bias	RPD	R^2_p	RMSEP	Bias	RPD
Raw spectra	447-1020	6	0.955	5.138	0.961	4.801	-0.629	5.094	0.922	6.734	-0.224	3.603
MSC		4	0.949	5.407	0.952	5.290	-1.437	4.623	0.904	7.489	-3.458	3,239
[a]1st Der.S-G		3	0.949	5.415	0.951	5.334	-0.802	4.485	0.909	7.297	-3.239	3.325
Raw spectra	447-702	4	0.847	9.462	0.813	10.446	0.807	2.341	0.831	9.921	-2.012	2.445
MSC		4	0.875	8.555	0.834	9.840	0.836	2.485	0.764	11.741	-4.125	2.066
[a]1st Der.S-G		6	0.903	7.539	0.824	10.145	-0.755	2.411	0.694	13.359	-6.381	1.816
Raw spectra	705-1020	4	0.899	7.658	0.903	7.529	-0.449	3.248	0.761	11.825	-3.005	2.052
MSC		3	0.893	7.919	0.906	7.403	-1.192	3.304	0.679	13.683	-10.015	1.773
[a]1st Der.S-G		4	0.878	8.426	0.902	7.558	-0435	3.236	0.534	16.491	-12.944	1.471

π: Wavelength, R^2_c: Coeffiecient of determination for calibration, R^2_v: Coefficient of determination for validation, R^2_p: Coefficient of determination for prediction, RMSEC: root mean square error for calibration, RMSEP: root mean square error for validation/prediction, RPD: ration of performance to deviation, MSC: multiplicative scatter correlation, 1st Der. S-G: first derivative Savitsky-Golay, [a]: 7-point smoothing, 2nd order polynomial.

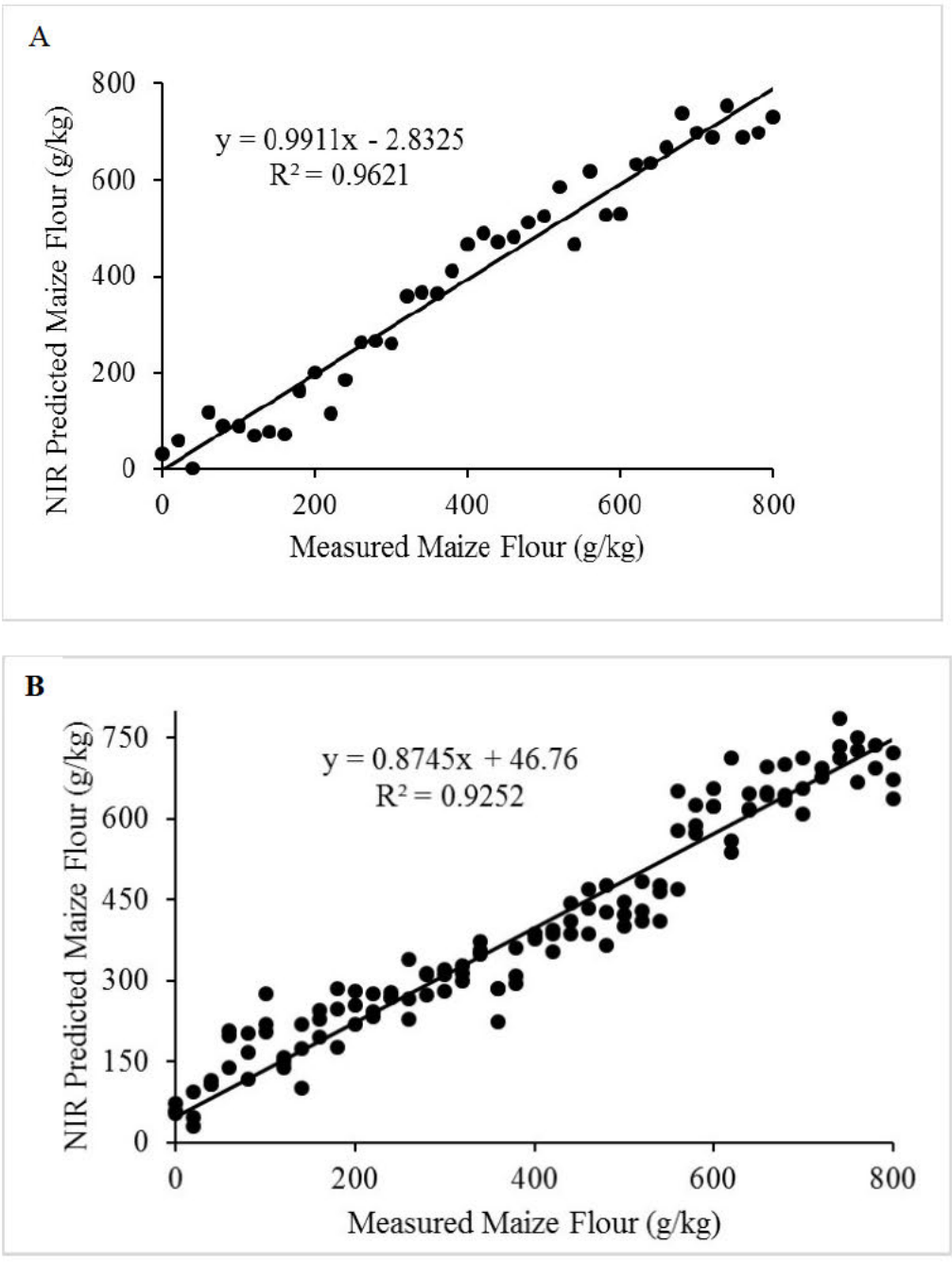

Figure 2: PLS validation (A) and test set (B) prediction plots illustration of positive correlation of NIR predicted vs measure maize flour contents in unripe banana flour after 1st derivative S-Golay 7-point smoothing, 2nd order polynomial.

4. Conclusion

The visible to near-infrared PLSR models developed in this research for the non-destructive prediction of maize flour adulteration in unripe banana flour was demonstrated to be accurate and showed stable robustness even when applied at a different environment mode. The handheld F-750 NIR quality meter appears to be a good tool in detecting unripe banana flour adulteration in processing factories. The PLSR evaluation enables the researcher to discriminate between pure and adulterated banana flour samples and also to quantify the level of adulteration with good consistent R^2 fit and satisfactory RPD. All studied wavelength regions were effective to give satisfactory prediction models. Therefore, portable NIR spectroscopy embedded with chemometrics is a potential method to be used in online monitoring and detection of adulteration and routine analysis of banana flour quality.

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CHAPTER 5 - SIMULTANEOUS PREDICTION OF UNRIPE BANANA FLOUR ADULTERATION WITH WHEAT (*TRITICUM AESTIVUM)* AND MAIZE (*ZEA MAYS)* FLOUR BY INFRARED SPECTROSCOPY AND MULTIVARIATE ANALYSIS

Abstract

Handheld visible-near infrared (Vis-NIR) spectroscopy is a novel and rapid tool that is used to quantify the adulteration of powdered processed foodstuff. This research was conducted to develop NIR spectroscopic models to predict adulteration of unripe banana flour with two less expensive staple flours, maize and wheat flours. Spectroscopic data was acquired using F-750 NIR spectrometer. Quantitative and qualitative analyses were performed on the 75% of dataset by leave-one-out cross validation approach through partial least squares regression (PLSR) and principal component analysis (PCA). Model performance was tested using the remaining 25% dataset for prediction. NIR models were developed after using several pre-treatment methods and compared based on maximum coefficient of determination of cross-validation (R^2_{cv}) and prediction (R^2_p), residual predictive deviation (RPD) and minimum root mean square error of prediction (RMSEP). PCA gave informative clusters between unadulterated and adulterated unripe banana flour samples against pure maize and wheat flour samples, showing 94% accuracy, with PC-1 and PC-2 accounting for 76% and 18% explained variance, respectively. First derivative Savisky-Golay (2^{nd} order polynomial with 9-gap smoothing) gave the optimum standard model, showing maximum R^2_{cv} (0.99), R^2_p (0.99), RPD_p (10.88) and minimum RMSEP of 2.42 g/kg. From the study, it was shown that the tested handheld F-750 NIR spectrometer a potential feasible tool that could rapidly detect and quantify adulteration of unripe banana flour. NIR spectroscopic models developed in this study could be employed to

simultaneously classify and predict maize and wheat flours unwanted adulteration to unripe banana flour product.

Keywords: Rapid screening; Product quality; First derivative; F-750 NIR spectrometer

1. Introduction

Unripe banana flour is one of the most valued commodities globally due to its gluten-free and resistant starch natural characteristics. It can be utilized as an ingredient in baked products, pasta and confectionaries (Adeniji, 2015). It is mostly processed in tropical and subtropical countries among which India, China, USA and Africa (such as Uganda and Tanzania) are leading producers (Padam et al., 2014; Chauhan and Jethva, 2016). The global banana flour market is estimated to reach a growth of US$735 million and consumption of 400 billion tons by 2027 (Future Market Insights Research, 2018). Approximately, 45 percent of total banana flour market revenues comes from the Middle, East and Western countries of Africa. In these regions, banana flour is popular and considered the primary source of carbohydrate (Ng et al., 2014; Joshi and Sarangi, 2014). A larger portion of its applications mostly goes to the food industry during the formulation of bakery, culinary, confectionery and beverage products (Adeniji, 2015).

The premium quality of powdered products like banana flour is crucial, especially for consumers, retailers, and processing industries involved in its importation and exportation. Moreover, the authenticity of unripe banana flour as a functional food is vital for both public health and economic gain. The preparation of powdered food is a complex agribusiness especially when large quantities of products to be processed involves different strict protocols

(Yolmeh and Jafari, 2017). During post-harvest operations, a lot of commodities become exposed to various types of adulteration (i.e. intentional and unintentional). In the presence of cheap products (Lohumi et al., 2015; Silvis et al., 2017), adulteration acts are easily performed. Unripe banana flour is included in the basket of noble and essential products of which temptations to easily be imitated with staple flours (e.g. wheat and maize flours) is common.

Generally, price is one of the factors consumers use to distinguish products quality between purchases (Mascarello et al., 2015). The processing of green banana flour in countries such as South Africa is new and a growing industry owing to an increasing number of the public adoptions on gluten free diets and healthy living standards. A price of a 1 kg of banana flour, ranges between $32 to $64 in local supermarket and health shops compared to staple flours such as wheat and maize which costs about $0.86 and $1.92 for a kilogram (kg), respectively (National Agricultural Marketing Council (NAMC), 2018; Stat SA, 2019). However, the price per kilogram varies between $10 - $80 (unripe banana flour); $0.77 - $1.96 (wheat flour); and $0.86 - $10.13 (maize flour) depending on the region (Global Price Info, 2020; The Statistics Portal, 2018). Another option that purchasers use is the description information about product ingredients displayed on the label (Olbrich and Christian, 2014). However, as specified by Mascarello et al. (2015), the use of these as indicators of product quality is inadequate and subjective when purchasing a product.

The differences in price range among commodities makes it vital for the agro-food industry, manufactures and retail market to adopt more objective tools with precision to make it possible to control food quality and to exchange their premium products at fair prices (Dabbene, 2014). Food processors and food inspection agencies can use a variety of vibrational spectroscopies

to guarantee unripe banana flour quality and monitor any sort of possible adulteration by unfair producers (Nenadis and Tsimidou, 2017).

Handheld near infrared (NIR) spectrophotometers are novel, recent on the market devices that food researchers, together with multivariate techniques, suitably utilise for the authentication and detection of adulteration of powdered food materials (Qu et al., 2015). The technique is user and environmentally friendly, non-destructive, rapid, and robust. Unlike the old age bench-top spectroscopies, handheld instruments allow manufactures to maneuver around with ease during the production process inspection. Moreover, in the food industry, NIR spectroscopy aids in controlling quality and quantity of food products (Sørensen, et al., 2016). In various literature, portable NIR tools have been proven to be a reliable method for the analyses of a variety of compounds present in different agricultural food products. For instance, Basri et al. (2016) studied the adulteration of lard in palm oil using portable NIR in combination with partial least squares regression (PLSR) and quantified the percentage of adulterant with 99% coefficient of determination (R^2) accuracy using transreflectance and transmission modes.

With F-750 NIR spectrophotometers (Felix instruments, WA, USA), information about the materials chemical structures is extracted empirically from the wavelength(s) (Wang et al., 2017). For a successful prediction model, adequate training data set along with ingredient of interest data, obtainable from a reference method, are required. In the process of model construction, chemometrics such as principal components analysis (PCA) and partial least squares regression (PLSR) are applied to give meaning to spectral data and extrapolate important features of materials studied (Correia et al., 2018). PCA is usually applied for classification analysis while PLSR is implemented for quantifying the ratios of adulteration. Once the prediction model is developed and tested for stability it can be installed back to the

instrument for future prediction of the characteristics studied (Basri et al 2016; Wang et al., 2017).

Quantification of adulteration in unripe banana flour has recently been investigated with visible-NIR spectroscopy indicating that the method is feasible and robust to predict staple wheat flour adulterant in unripe banana flour (Ndlovu et al., 2019). NIR technologies application to control food fraud are believed to miniaturize diverse false presentation of products. Moreover, the use of portable NIR technologies makes it convenient to monitor and regularly check product authenticity at pack-houses, prior off-loading at supermarkets and directly on shelf (Schmutzler and Huck, 2016).

In this work, NIR F-750 spectroscopy in conjunction with chemometrics is evaluated to provide a simultaneous qualitative and quantitative detection of maize and wheat flours adulteration in unripe banana flour. Developing standard NIR based model(s) to discriminate unripe banana flour adulteration from South African staple flours will be a quick and efficient approach for commercial scale producers. Given that NIR devices are one of the green technologies, that is, easy portability, no chemicals nor continuous sample preparations are required during the analysis. The work reported herein was to determine an NIR F-750 spectroscopy can be used to predict varying degrees of both maize and wheat flours adulteration in unripe banana flour. This was achieved through comparing NIR based pre-processing methods by (a) evaluating the F-750 NIR tool capability to discriminate maize-wheat adulterated banana flour samples by PCA and (b) quantifying adulteration concentrations for both adulterants in banana flour through PLSR analysis.

2. Materials and methods

2.1. Preparation of samples

2.1.1. Composite unripe banana flour

The green (unripe) banana fruit were provided by the Agricultural Research Council-Tropical and Subtropical Crops (ARC-TSC), Burgershall Research Station, South Africa (25° 6′0″ S, 31° 4′60″ E). Using the standards banana colour chat by SH Prattt & Co (Bananas) Ltd (Luton) (Tapre and Jain, 2012); twenty-two cultivars utilised in this study were determined fully matured green banana fruit at stage one of ripening. The harvested bananas used for the study were a collection of diploids, triploids and tetraploids *Musa*-genotypes as shown in Table 1. The unripe banana flour developed for this research was prepared entirely from the pulp of fruit not subjected to any ripening following the method by Ndlovu et. al (2019). The blending of banana flour cultivars to formulate a composite banana flour was introduced in this research as the concept emphasized and initiated by the Food and Health Organization (FAO) 1964 (Fellers and Bean, 1998). Concisely, the composite flours were implemented as an approach of using uncommon food products intended to replace wheat flour with novel functional properties for human nutrition (Noorfarahzilah et al., 2014). The development of composite green banana flour is ideal for developing countries like South Africa to increase production and supply of gluten-free and high resistant starch food products to meet health demand of various individuals. It also establishes a foundation to encourage the utilization of locally grown native banana fruits as flour by farmers and individual households.

2.1.2. Maize and wheat flours

Maize flour (MF) was prepared from rough or coarse grounded dried white maize grains (Iwisa maize rice, Premier Foods, (Pty) Ltd, South Africa), purchased from a local supermarket and

milled using a laboratory machine (S8 Range, Drotsky Aktief (Pty) Ltd, SA). Wheat flour (WF) (SASKO cake wheat flour, Pioneer Foods (Pty) Ltd, South Africa) sample were purchased from a local supermarket. Prior adulteration experiments and spectra acquisition, unripe banana and maize flours were further filtered (repeated 3 times) in a 355-mesh sieve (Universal laboratory test sieve, SABS, SA) to ensure particle size uniformity. Approximately 200 g/kg unripe banana flour was weighed (Electronic Balance (BL-3200H), Shimadzu Corporation, Japan), and decanted into airtight plastic mixing container where subsequent adulterant levels of (20-800 g/kg) maize flour were added and thoroughly mixed.

Table 1: Banana varieties used to prepare composite unripe banana flour

Species	Cultivar Name	Genome Group
M. acuminata (AA) x *M. acuminata* (AA)	Chinese Cavendish	AAA
	Gros Michel	AAA
	Grand Negra	AAA
	Valery	AAA
	Williams	AAA
	D11*‡	AAA
	MCC*‡	AAA
	Calcutta 4	AAA
	Sordwana*‡	AAA
	IPB5-61	AAA
	Green Red	AAA
	Khuai Thong Raung	AA
M. acuminata (AA) x *M. balbisiana* (BB)	PK6*‡	AAAB
	Fhia-01	AAAB
	Fhia-18	AAAB
	Hinoon	AAAB
	Gold Finger	AAB
	Lady Finger	AAB
	Prata Anna	AAB
	Pome	AAB
	Foconnah	ABB
	Ducasse	ABB

*‡ ARC-TSC selections.

Source: Perrier and Tézenas du Montcel, (1990).

2.2. Measurement of NIR spectra

A 5 g (± 1) sample of unadulterated and adulterated mixtures was weighed (Electronic Balance (BL-3200H), Shimadzu Corporation, Japan) in triplicates, placed individually into petri dishes (50 mm x 55 mm), and enclosed in machine sample holder. In reflectance mode, spectra of pure maize, wheat and unripe banana flour and the subsequence adulterated UBF samples were collected using the F-750 NIR spectrometer (Felix Instrument, WA, USA). The instrument was equipped with a Xenon Tungsten lamp. It recorded each spectra measurement at 3 nm interval sampling in the range of 315-1200 nm with spectral resolution of 8-13 nm. Spectra of pure samples as well as adulteration mixtures were acquired as shown on figure 1 below. A total of 110 spectra were obtained. The acquisition of data was managed by the F-750 Data Viewer software (Version 1.2.0.75, Felix Instruments, WA, USA). A common characteristic when working with NIR devices is that noise is normally experienced at the beginning and end of the spectra. As noise was noted in this study, the initial range of 315-1200 nm was trimmed and an NIR wavelength zone between 444-1026 nm was used for analysis.

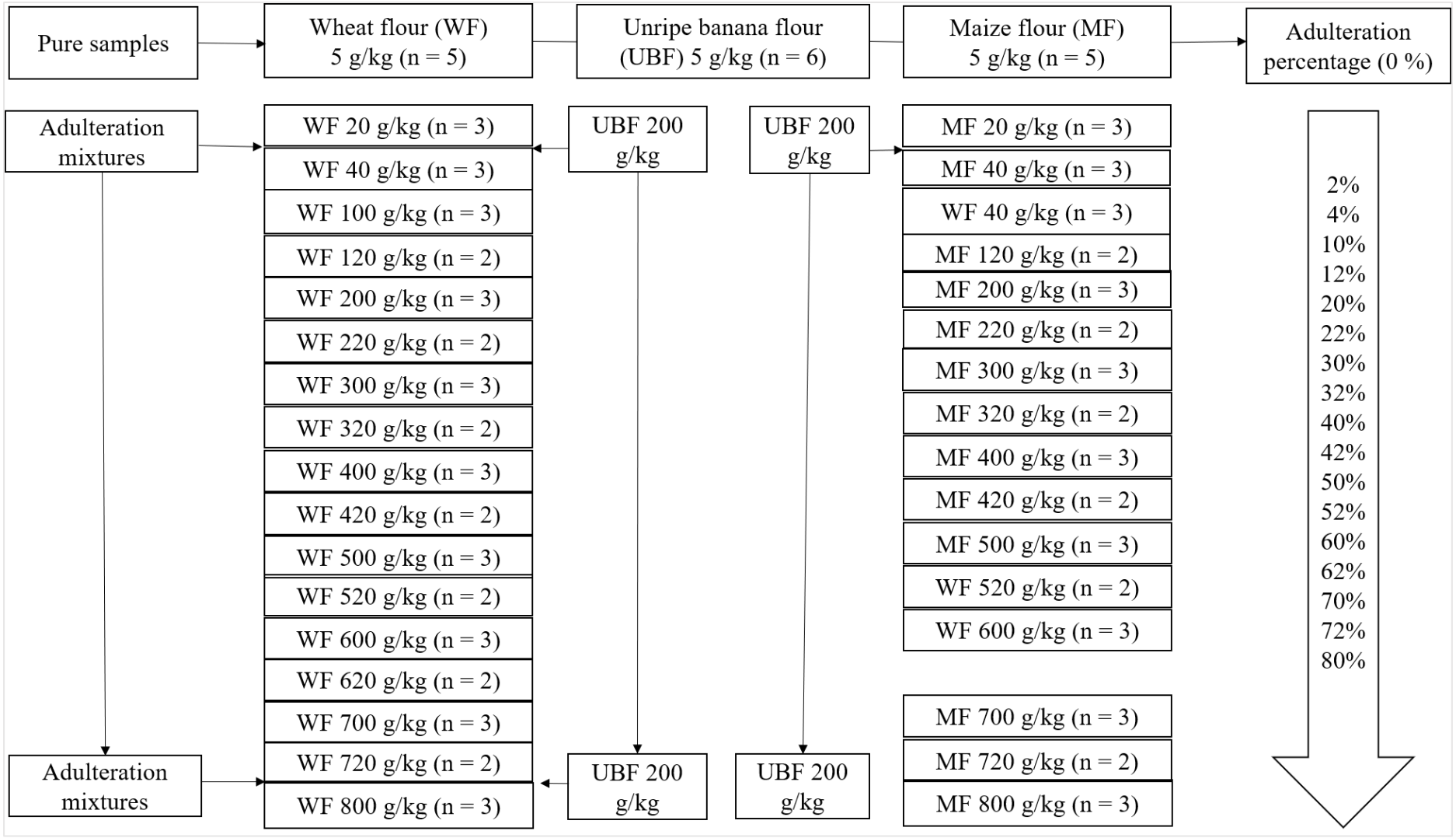

Figure 1: Setup flow diagram of adulteration mix experiments and spectra acquisition

2.3. Multivariate data modelling

2.3.1. Principal component analysis (PCA) and partial least squares regression (PLSR)

The obtained spectral and reference data sets of pure and adulterated unripe banana flour samples were analysed by partial least squares regression (PLSR) and principal component analysis (PCA). These two chemometric techniques are powerful quantitative and qualitative multivariate tools useful to describe the fundamental relationship between spectral dataset and analyte concentrations. Moreover, they are responsible for minimising dimensionality of collinear variables. PCA was conducted on the input data to discriminate and partition samples according to spectral variation with respect to adulterants and their subsequent adulteration levels (mentioned above) through non-linear iterative partial least squares (NIPALS) algorithm.

PLSR is adequate for expression of the linear mathematical combinations between spectra and the reference parameters (Morsy and Sun, 2013). It involves the decomposition of independent spectral data into latent variables describing the maximum covariance between spectra and contents of parameter of interest (Sunoj et al., 2016). The quantification of adulterants concentration was analysed by orthogonal partial least squares logarithm. This log filters noise in the dataset, and reduces the model complexity by lowering the number of latent variables (LVs) in addition to allowing the identification analysis and investigation of the main source of samples variation (Word et al., 1998). The lowest number of latent variables holds relevant information regarding the prediction model and also is an indicator of the optimum models efficiency. Calibration models were constructed by leave-one-out cross-validation method.

The cross-validation approach allows an opportunity of a calibration model to estimate the expected level of fit of a model to a dataset that is independent of the data that were used to train the model (Mabood et al., 2017).

The concept of leave-one-out cross validation method is that each spectrum or sample gets to be omitted from the analysis as the remaining dataset are used to build and predict a model. This process of omitting spectrum is repeated until all samples in the training dataset are being used for calibration and validation to give a complete series of predictions for the whole dataset. Seventy-five percent of the data was assigned to develop calibration models (n = 65). The resultant calibration models were tested for performance on the 25% remainder of spectral dataset (n = 43). All quantitative and qualitative analysis were performed on mean centred data executed by the Unscrambler X software (The Unscrambler X Version 10.3; CAMO, Trondheim, Norway).

2.3.2. Chemometrics spectral transformation for quantifying adulterants

Various chemometrics pre-processing methods were performed on spectral data to address undesirable effects resulting from external factors such as particle size variation among samples, light scatter, random noise and to smooth spectral data. The untreated spectra were compared against the original 1^{st} and second derivatives, 1^{st} and 2^{nd} derivatives (Savisky - Golay logarithm, second polynomial) with 9 and 13 smoothing gaps, respectively; multiplicative scatter correction (MSC) and standard normal variate (SNV). Their combinations (1^{st} Deriv + SNV + MSC and 2^{nd} Deriv + SNV + MSC) were evaluated to optimise on model prediction performances. In each case a new PCA classification was examined and a new PLSR model was developed, results were evaluated.

2.3.3. Assessment of PLSR adulteration prediction models

The best PLSR models were estimated based on the highest coefficients of determination value for cross-validation (R^2_{cv}), prediction (R^2_p) and residual predictive deviation (RPD); and lowest root mean square error of cross validation (RMSECV) and root mean square error of prediction (RMSEP) as illustrated on equations 1-4. (Saeys et al., 2005; Jiang et al., 2015)

$$R^2 = 1 - \frac{\Sigma(y_{cal} - y_{act})^2}{\Sigma(y_{cal} - y_{mean})^2} \quad (1)$$

$$RMSECV = \sqrt{\Sigma(y_{cal} - y_{act})^2}/n \quad (2)$$

$$RMSEP = \sqrt{\Sigma(y_{pred} - y_{act})^2}/n \quad (3)$$

Where y_{cal} is the calculated value, y_{act} is the actual measured value, y_{pred} is predicted value of the adulterant concentration, y_{mean} is average value of predicted data; and n is number of spectra.

$$RPD = \frac{SD}{RMSEP} \quad (4)$$

where SD is the standard deviation of reference measured adulterant concentrations.

3. Results and discussion

3.1. Individual spectra characterization of pure flour samples

The F-750 NIR spectroscopy is a rapid and a sensitive tool that could be utilized to rapidly screen quality of unripe banana flour during industrial application, since its ability to differentiate between the pure wheat and maize flours was possible even before they were merged at varying adulteration concentrations (Figure 1). The diagnosed spectral differences of pure samples in the visible to near infrared region (444 - 1026 nm) of this study associates with overtones or combinations of fundamental stretching bands (Agelet and Hurburgh, 2010). There was an overlap of sharp absorption intensities at the visible region. Wheat flour (WF) exhibited the most prominent peaks at 438 nm, 498 nm and 685 nm, pure maize flour (MF) had notable peaks at 464 nm and 531 nm, while unripe banana flour (UBF) spectrum showed intermediate band at 498 nm. Other peaks at 608 nm, 626 nm, 746 - 842 nm, 865 nm, 885 nm, 908 nm 938 nm and 967 nm were observed and maintained a similar wavelength pattern with varying absorption wavebands between pure samples. The spectrum of each pure sample varied and this difference could be assigned to the flours surface traits and unique biochemical constituents (Sunoj et al., 2016).

3.2. Spectra of the adulterated samples

The spectra for wheat and maize adulterated UBF samples observed on Figure 2 also showed that the adulterated samples had a similar trend in absorbance. The obtained differences in absorption peaks can be attributed to the stretching of hydrogen groups -O-H-; and -N-H- of the samples and vibrations from -C-H-; first and second overtones as well as second overtones of -C=O- groups and the combination of amides and amines (Osborne, 2006; Sunoj et al., 2016).

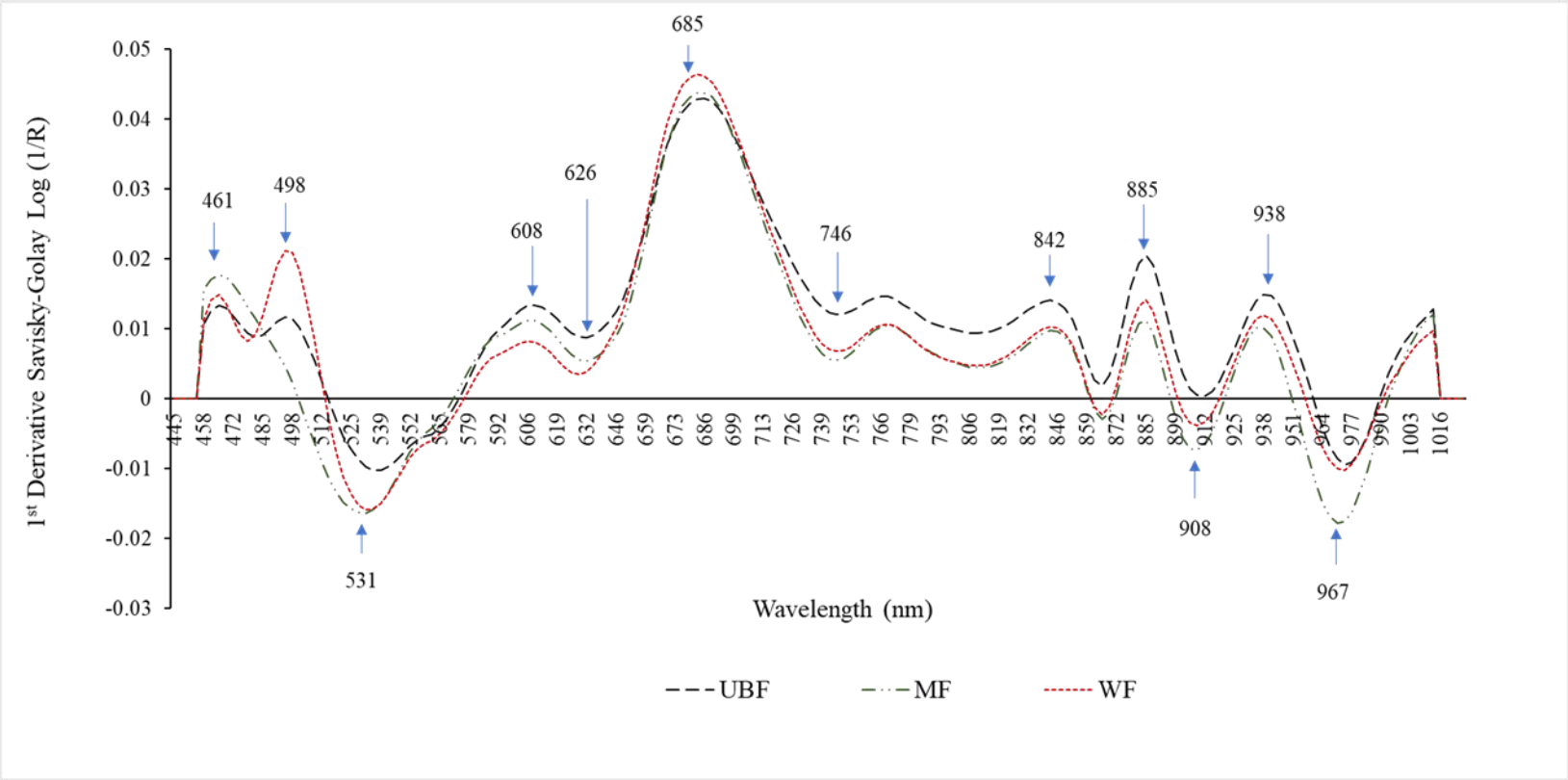

Figure 1: Typical wavelength spectra of differences between unadulterated samples

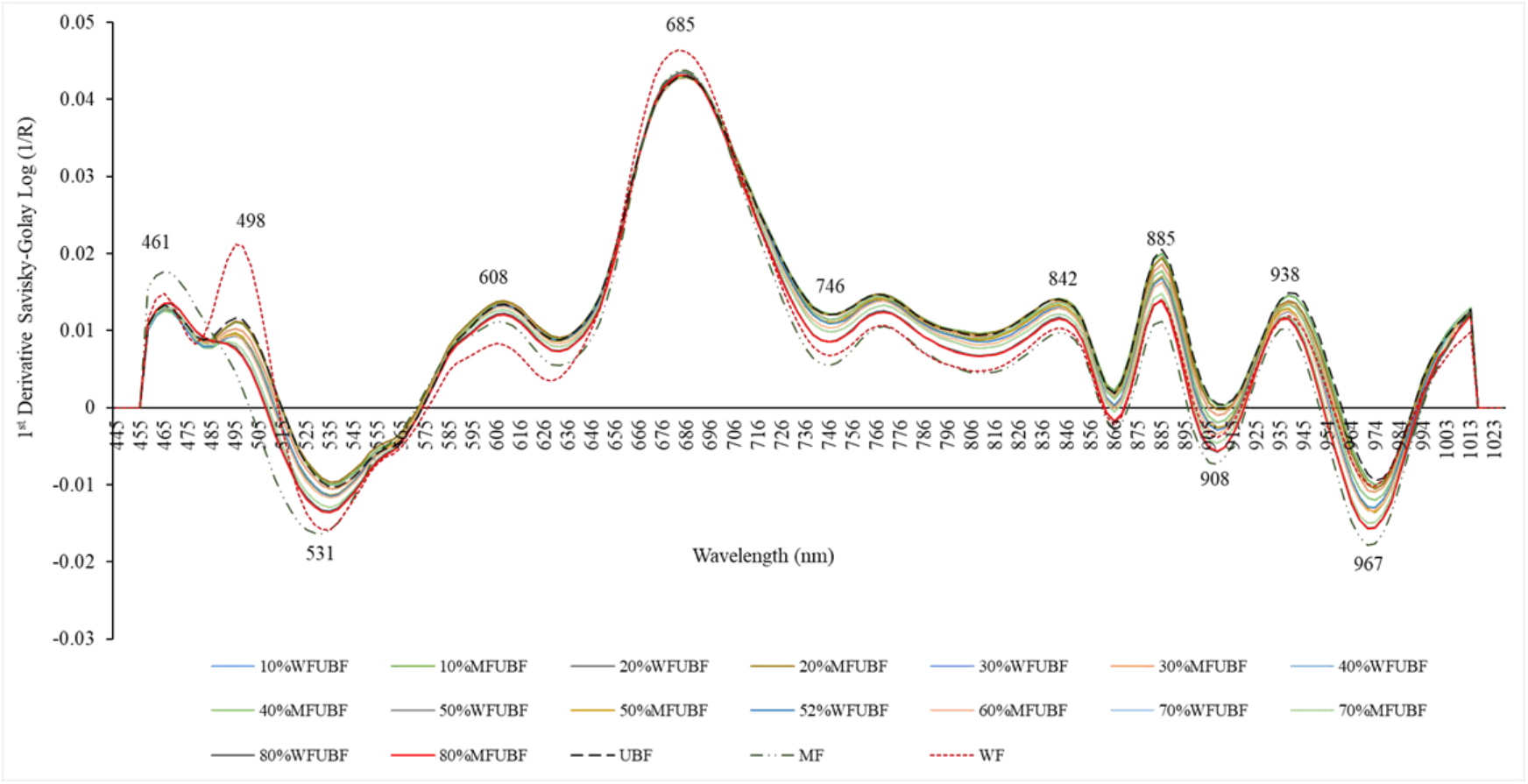

Figure 2: All spectra showing pure flours before mixing and adulterated unripe banana flours with different pure wheat and maize flour levels.

3.3. Principal Component Analysis model

PCA is an un-supervised chemometrics method which aids to explore and visualize spectral data into clusters. This technique diminishes dimensionality issues of spectral matrix in a manner that correlate a set of variables into reduced dimensional positions (called principal components (PCs)), thus, displaying data trends containing the most relevant information of food materials (Kiers et al., 2007; Singh et al., 2010). First derivative Savisky-Golay (2^{nd} order polynomial, 9-smoothing gap points) transformed the original spectra and removed background shift, giving simultaneous discrimination of pure samples and corresponding concentration mixtures of adulterants.

The PCA modelling showed a 94% diversity between samples for the first two principal components mapping twelve categories among which three groups are for pure flour samples and the remaining nine are adulterated mixture samples (Figure 2). From the PCA plot, pure banana flour samples are positioned on the positive component loadings on PC1 while cluster relating to pure maize and wheat flour samples are located on the oppositive negative component loadings. Samples with lower concentration (2-4% and 10-12%) of adulterants were close to unadulterated banana flour samples. This observation could imply that it would not be easy to distinguish adulterated banana flour by visual inspection alone. Hence, the requirement to implement NIR spectroscopy for adulteration monitoring routines. Moreover, as the level of adulterants increased (20-22% until 80%) the diversity between samples was getting more visibly spread towards pure cereals flour samples.

The loadings plot of PC-1 and PC-2 of all samples (Figure 3) showed wavelength peaks that were the most informative and characteristic in the current adulteration investigation. The most visible bands on PC1 (515 nm, 676 nm) and PC2 (498 nm, 676 nm) were assigned to the

stretching of –C=O-H- and -C=C-C-, attributed to the carbonyl aromatic and benzene ring compounds (Coates, 2006). The bands from 742 to 961 nm (PC1) and PC2 (970 nm) can arguably be correlated to second overtone of hydrogen stretch and –N-H-, amides and amines; and third overtone stretching of –O-H- and –C-H- organic groups of starch carbohydrates (Stewart, 2004; Osborne, 2006).

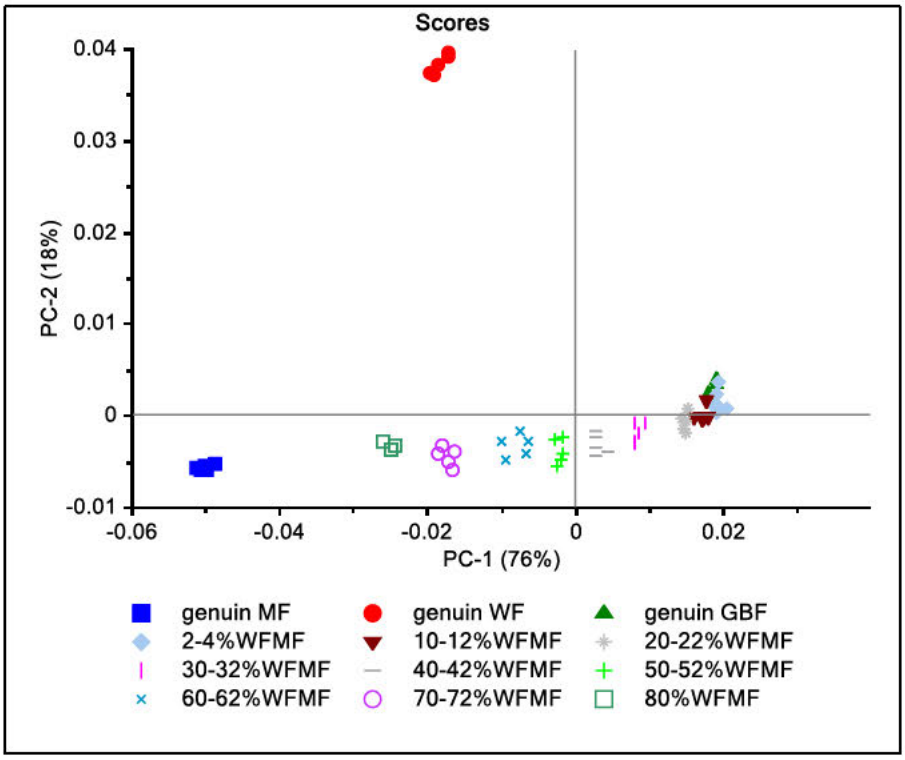

Figure 2: Principal components scores of adulterated and unadulterated samples.

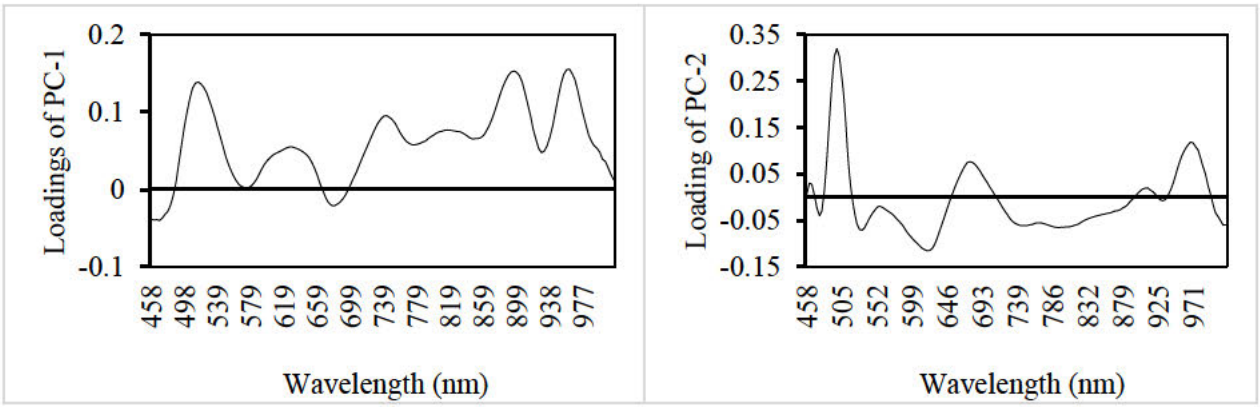

Figure 3: The loadings plot from PC1 and PC2 of all samples.

3.4. PLSR adulteration prediction

Table 2 compares statistical indexes of models obtained with different data pre-treatments. Depending on spectral data pre-processing method, models generated in the study gave good fit with satisfactory predictive R^2 values and low RMSE values acceptable for developing calibration and prediction models. The predictive models generated resulted in RPD values higher than 3. This was an indication that these models could be considered excellent and most reliable for analytical prediction of wheat and maize flour adulteration in unripe banana flour. The prediction model developed using 1^{st} derivative Savisky-Golay with 9 smoothing points (2^{nd} order polynomial) was observed to give the highest R^2 for prediction (0.99), lowest detection limit (RMSEP) of 2.42 g/kg and highest RPD value of 10.88 (Table 2).

The pre-treatment of spectral data is essential and in some other scenarios need to be optimized individually by combining pre-processing methods in order to achieve better accuracy and prediction reliability. The calibration models developed with original 2^{nd} derivative had highest number of factors, highest RMSECV (11.5 g/kg) and lowest RPD (1.4) values and this model was considered not fit for the assessment of unripe banana flour adulteration. Subsequently, further smoothing of the original derivatives and merging them with other pre-processing methods turned out to be of significance importance and this resulted into improve prediction ability of the models (Table 2). Considering these findings, it can be portrayed that the independent test set prediction models in Figure 4 has good performance in predicting maize and wheat flour adulteration in unripe banana flour.

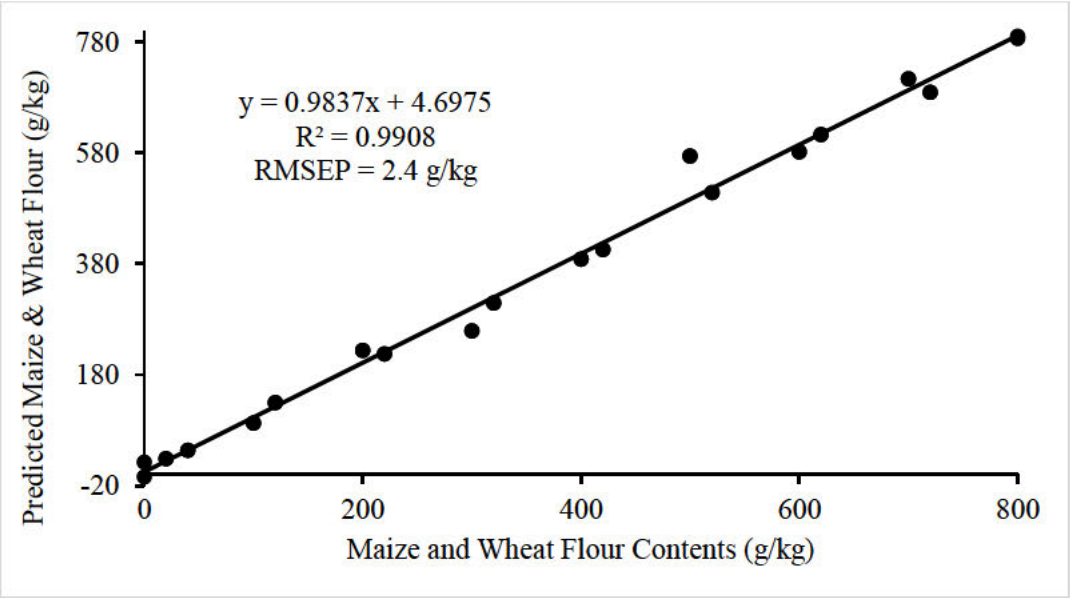

Figure 4: Scatter plots display of NIR predicted versus measured reference wheat and maize flours adulteration contents.

Table 2: PLSR prediction models and the influence of different transformation methods on calibration models performance

Pre-treatment	Factor	R^2_c	RMSEC	R^2_{cv}	RMSECV	R^2_p	RMSEP	RPD	Bias	Slope
Original spectra	3	0.993	2.251	0.992	2.418	0.991	2.325	11.305	0.228	0.992
Original 1st Der.	2	0.989	2.695	0.983	3.466	0.985	3.117	8.432	-1.228	0.970
Original 2nd Der.	**7**	**0.988**	**2.901**	**0.812**	**11.498**	**0.483**	**18.069**	**1.455**	**0.144**	**0.589**
SNV	2	0.985	3.153	0.983	3.480	0.977	3.811	6.897	0.339	0.964
MSC	2	0.985	3.223	0.983	3.483	0.977	3.849	6.829	0.336	0.963
1st Der. 9-pt	**2**	**0.991**	**2.489**	**0.989**	**2.687**	**0.991**	**2.416**	**10.881**	**-0.127**	**0.984**
2nd Der. 13-pt	1	0.969	4.574	0.968	4.755	0.980	3.597	7.307	-1.549	0.955
MSC+SNV+1st Der. 9-pt	**1**	**0.990**	**2.645**	**0.989**	**2.736**	**0.991**	**2.425**	**10.840**	**-0.434**	**0.988**
MSC+SNV+2nd Der. 13-pt	**1**	**0.977**	**3.962**	**0.976**	**4.113**	**0.985**	**3.120**	**8.424**	**-1.290**	**0.970**

R^2_c: coefficient of determination for calibration; RMSEC: root mean square error of calibration: R^2_{cv}: coefficient of determination for cross-validation; RMSECV: root mean square error of cross-validation; R^2_p: coefficient of determination for prediction; RMSEP: root mean square error of prediction, RPD: residual predictive deviation; 1st Der.: first derivative Savisky-Golay; 2nd Der.: second derivative Savisky-Golay; MSC: multiplicative scatter correlation; SNV: standard normal variate.

4. Conclusion

The obtained results demonstrated spectral pre-processing significantly enhances the predictive performance of the models. Excellent calibration, cross-validation and prediction models generated in the current research confirms that the F-750 handheld near infrared spectroscopy together with chemometrics holds a promising role to the food industry as a real time tool to screen for unripe banana flour adulteration with wheat and maize staple flours. In conclusion, the robust prediction models developed in this study are believed to be suitably applied to control banana flour quality by industry and retailers' prior distribution and shelf display.

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CHAPTER 6 - RAPID SPECTROSCOPIC METHOD FOR QUANTIFYING GLUTEN CONCENTRATION AS A POTENTIAL BIOMARKER TO TEST ADULTERATION OF GREEN BANANA FLOUR

Abstract

The demand for gluten-free banana flour has led manufactures to enforce strict measures for quality control. A need has arisen for the development of more sensitive and reliable methods to test the quality of green banana flour (GBF). The objective of this study was to develop rapid visible to near-infrared (Vis-NIR) based spectroscopic calibration models to quantify gluten concentration, as a biomarker to detect wheat flour adulteration in green banana flour (GBF). Spectroscopic data were acquired using a desktop (FOSS®) Vis-NIR spectroscopy ranging from 400 to 2500 nm of the electromagnetic spectrum. The spectral and reference data were submitted to partial least squares regression (PLSR) for the development of gluten adulteration detection models. Calibration models were constructed based on a full cross-validation approach, consisting of 51 samples for the calibration set and 21 samples for the test set. The optimal prediction model was obtained after a combination of baseline (offset and baseline linear correlation) and standard normal variate (SNV) pre-processing technique. This model showed a 94% coefficient of determination of cross-validation (R^2_{cv}) and prediction (R^2_p); root mean square error of cross-validation (RMSECV) of 3.7 mg/kg, root mean square error of prediction (RMSEP) of 3.9 mg/kg; and RPD value of 4. This work has demonstrated that Vis-NIRS method is a robust and feasible technology that may be used to ensure the safety of banana flour and that this product stays gluten-free by providing good and reliable gluten detection and quantification prediction models.

Keywords: Non-destructive technology; gluten prediction; partial least square regression; product safety; consumer protection

1. Introduction

Green banana flour (GBF) is one of the important horticultural products in global trade. It is a significant component in the diet of many African populace as well as other international countries such as China, the United States, Europe and India, to mention a few. A considerable growth in the market for gluten-free products is estimated to reach $7.91 billion by 2026 from $4.35 billion in 2018 (Global Market Insight, 2019). As a result of the significant research in previous years which generated information about gluten-free products has allowed food processing firms to expand in competition to offer a high number of foodstuffs with gluten-free claims (Witczak et al., 2016). Currently, powdered bananas are preferred for their superior nutritional quality, diversity taste and applications to various dishes or products and their health benefits to reduce chronic illnesses (Ranjha et al., 2020).

It is no doubt that the expansion of green banana flour processing has stimulated competition among the producing countries. Nowadays, it is inadequate to view emerging food products only on the proximate components (*viz* proteins, minerals, moisture content, etc.). However, consumers need to be aided with specific relevant important knowledge and awareness of the benefits representing the foods they eat (Başlar and Ertugay, 2011).

In that regard, natural biomarkers are important quality food fingerprints to consider during the manufacture and processing of any foodstuff (Malheiro et al., 2013). They can be mainly used as

a precise indicator of the nutritional status of food products (Pico et al., 2019) and could act as a guide to modernized consumers which assist to make informed decisions during purchases (Medina et al., 2019). The identification of molecular biomarkers would be an excellent area for research investigation with regards to green banana flour. It would provide producers with more effective means to maintain the products' nutritional quality as well as to be able to screen and detect possible food contamination exposure and food adulteration incidents (Medina et al., 2019). Various reports in the literature claim GBF is a natural 'gluten-free' product (Singh et al., 2016), thus, a distinct novel biological characteristic. Concisely, to conform to this claim a gluten-free product such as GBF, according to the Codex Alimentarius Standard 118-1979 (2008), are dietary foods with a gluten level not exceeding 20 mg/kg irrespective of whether the product contains no wheat, barley, rye and their crossbred varieties or especially have been formulated to eliminate gluten.

However, gluten-free powdered foodstuff including GBF may become easily contaminated at any point during the production cycle with physical or chemical materials that should not be included owing to shared processing equipment (Erkinbaev et al., 2017). Due to manufacturers increased awareness of the growth in the number of consumers willing to pay premium prices for gluten-free products, it is also practically possible that a product such as GBF may be subjected to false labeling for either careless or illegal adulteration reasons (Guelpa et al., 2017). A recent research study revealed that commercial food products in developing countries such as South Africa may have misleading and insufficient labelling information with regards to their wheat allergens or gluten claims (Cawthorn et al., 2010).

Gluten is a complex protein that is naturally found in powdered cereal grains such as wheat flour. It is made up of two components, namely, gliadin and glutenin, which give wheat flour its viscosity and elastic properties that are responsible for sensory traits based on several baked products (Haraszi et al., 2011). However, wheat gluten protein is also a common allergen of which a significant proportion of the population around the world is intolerant (Czaja et al., 2016). Particularly in the Western regions, approximately 0.6 to 1% of the population is genetically susceptible to wheat and gluten-containing products (Falcomer et al., 2020). Furthermore, wheat gluten is capable of inducing a wide range of adverse allergies and disorders. For example, allergies such as bakers' asthma, wheat-dependent exercise-induced anaphylaxis and atopic dermatitis may occur via inhalation or skin contact due to physical occupational exposures with wheat flours (Rongfei et al., 2014). In addition to that, the intake of gluten-rich foods has been reported to induce celiac disease, which affects both adults and children at various rates from 0.1 to >1.6% (Abadie et al., 2011; Rosell et al., 2014).

Celiac disease is an immune-mediated chronic inflammatory of the small intestine, which typically results in nutrient malabsorption (Haraszi et al., 2011; Chu et al., 2012). Consequently, giving rise also to secondary illnesses such as anemia and vitamin deficiency (Nassef et al., 2008), amongst many. Until to date, there has been no reported cure for celiac disease (Cawthorn et al., 2010). However, the only effective therapy for sufferers is the lifelong exclusion of gluten and subsequent products (Allred et al., 2010). This makes a gluten-free product as GBF an essential and desirable product in the diet of individuals with the above-mentioned gluten intolerance conditions as well as consumers who, as a lifestyle choice, choose to stick to a gluten-free diet (Almeida-Junior et al. 2017).

Wheat gluten adulteration diagnostics requires countries to put in place strict protocols to monitor what is produced locally and shipped inside as imports. This also implies that manufactures of gluten-free GBF should be cautious and practice running frequent quality inspection routines against wheat contamination to safeguard sensitive individuals and to protect their business profiles (Cawthorn et al., 2010; Erkinbaev et al., 2017). To avoid serious health complications, people suffering from gluten-induced conditions primarily rely on accurate food labelling to make good choices during purchasing (Jabri et al., 2005). Therefore, producing high-quality gluten-free GBF is of high socio-economic importance and the scientific approach to assess and quantify its adulteration by wheat gluten is a significant necessity. Various analytical methods have been developed to facilitate the testing or monitoring of gluten adulteration in gluten-free products. The Codex Alimentarius Committee (2008) declared several types of enzyme-linked immunosorbent assay (ELISA), which are the commercial destructive methods for the analysis of gluten in cereal and pseudo-cereal derived products and many as described by Haraszi et al. (2011). Researchers have also established other classical methods such as liquid chromatography-mass spectroscopy (LC-MS) (Lock, 2014) and polymerase chain reaction (PCR) (Mujico et al., 2011) for the detection of undeclared wheat allergens in gluten-free food products.

However, these analytical approaches are time-consuming, laborious, need chemical reagents, and trained staff to perform the analysis (Chu et al., 2012). In this regard, visible to near-infrared spectroscopy (Vis-NIRS) technology combined with chemometrics is a fast, robust, sensitive and environmentally friendly method (Wang, 2019). It has been used to screen for quality and quantify wheat gluten in several adulterated agricultural food samples (Ahmad et al., 2017). Although GBF

has been researched for its phenolic and antioxidant capacities (Sarawong et al., 2014), physicochemical, pasting and textural properties (Flores-Silva et al., 2015). To our knowledge, Vis-NIRS has been little explored for the detection of gluten adulteration in GBF. Therefore, to fill this knowledge gap, the main objective of this study was to evaluate the potential of Vis-NIRS coupled with partial least squares regression (PLSR) for detection and quantification of GBF adulteration by wheat gluten.

2. Material and methods

2.1. Pure and adulterated samples preparation

All banana fruit were provided by the Agricultural Research Council - Institute for Tropical and Subtropical Crops (ARC-TSC), South Africa. Table 1 shows the banana genotypes utilized in this research to generate green banana flour samples. Wheat flour was already prepared and was obtained from a local supermarket. The green banana flour developed for this research was prepared entirely from the pulp of fruit not subjected to any ripening at stage I (Tapre and Jain, 2012). Prior to peeling and slicing of pulp, all banana fruit samples were rinsed with water to remove farm debris.

Banana fruit pulps were dried for 15 hours at 50 °C in a hot air dehydrated (AD3000 Agri-Dryer, Dryers for Africa, Limestone Hill, South Africa). The grinding of samples into flour was carried out in a laboratory-scale electric miller with 0.8 mm size sieve (S8 Range, Drotsky Aktief (Pty) Ltd, South Africa). For a finer particle size distribution filtered all pure flours were filtered in a 355 mm sieve (Universal Laboratory test sieve, SABS, South Africa). The blending of banana

flour from different cultivars to formulate a composite banana flour was adopted in this research as the concept emphasized and initiated by the Food and Health Organization (FAO) 1964 (Fellers and Bean, 1998). For uniform particle size distribution, before the mixing of samples and spectra acquisition. In two set of experiments, the combinations of adulterated 20 g GBF samples were prepared by mixing wheat flour at levels of 0%, 2%; 10%, 20%, 30%, 40% 50%, 60%, 70%, 80%, 90% and 100%, with three samples at each level. A total of thirty-nine sample batches which consisted of thirty-three adulterated samples, three pure GBF and three wheat flour samples were prepared for wet analysis and near-infrared spectroscopic study. The prepared samples were stored at -20 °C for further analysis.

2.2. Acquisition of Vis-NIRS spectra

A laboratory bench-top monochromator NIR Systems Model XDS spectroscopy (Foss NIR Systems, Inc., Maryland, USA). The instrument was facilitated with a quartz halogen lamp and lead sulfur (PbS) detector, used to measure the reflectance spectra of samples. To reduce the influence of instrumental shifts, the NIRS instrument system was calibrated with a one hundred percent white reference tile before and after every 30 minutes in between sample measurements. Spectra of all homogenous samples (5 grams) were acquired with a round cup sample holder with a quart glass (38 mm diameter and 10 mm in thickness) which was placed in the instruments' enclosed compartment to prevent light escape. The NIR system was operated with Vision software (Vision TM, version 3.5.0.0, Tidestone Technologies Inc., KS, USA). Using a full wavelength range (400-2498 nm) spectra were collected at 2 nm interval. Each recorded sample spectrum consisted of 32 scans which were then automatically averaged and stored as log (1/R); where R represents reflected intensity. The acquisition of spectra was done in duplicate.

2.3. Chemical analysis

2.3.1. Pure sample reference characteristics

To ensure there was no cross-contamination between raw samples, in this study pure samples of unripe banana flour and wheat flour were initially measured for their common proximate profile. All raw samples were analyzed in triplicates. The moisture contents (MC %) of green banana flour and that of wheat flour samples were determined to be 7.83% and 8.62%, respectively (Horwitz and Latimer, 2006). Pure flours were determined for their crude protein by the Dumas combustion (AOAC official methods (1980). Briefly, 2 g of samples were weighed in ceramic boats and added with 2 grams of EDTA catalyst. The samples were loaded into Leco instrument analyzer (Leco, corporation, USA) initiated with TruMac software (Leco, corporation, USA). The crude protein content was calculated by multiplication of nitrogen % of each sample by 6.25.

2.3.2. Gliadin/gluten extraction and quantification by enzyme-linked immunosorbent assay (ELIZA)

The R5 ELISA RIDASCREEN Gliadin R7001 (R-Biopharm, AG, Darmstadt, Germany) test kit was used for the extraction and confirmation of gliadin and/ gluten-free properties in pure GBF samples. In addition to that pure wheat samples and GBF samples with corresponding wheat flour contaminations were quantified for the presence of gluten contents. The R7001 is an effective quantitative method for the analysis of gliadin and gluten antibodies from cereals such as wheat, rye and barley. The test kit has the capability to assess the quality of ‘very low gluten’ and ‘gluten-

free' declared powdered foods. Samples were extracted following the guidelines of the manual as provided by the manufacture of the test kit.

Briefly, in corning culture tubes (16 x 125 mm), homogenous samples of 0.25 g were extracted with 2.5 mL cocktail patented (R7006) and incubated for 40 minutes at 50 °C. The samples were then treated with 7.5 mL of 80% ethanol-distilled water, shaken upside down in a rotator for about an hour at 20 ± 5 °C room temperature (RT). The sample extract (2 mL) was centrifuged in a high-speed microcentrifuge (2500 g) for 10 minutes. The supernatant of 80 µL was diluted with the gliadin diluent (920 µL) and used immediately for the analysis. A 100 µL of the sample solutions, were pipetted to the microtiter plate wells in duplicates. Each well coated with R5 antibodies to recognize and capture gliadins of the samples, forming an antibody-antigen complex. Post incubation of 30 minutes at RT, the pipetted sample solution was discarded and wells washed thrice with a 250 µL washing buffer with vigorous upside down tapping of the microtiter plate against absorbance paper towel, to ensure removal of liquid from the wells. Freshly prepared conjugate (100 µL) was added to each well and incubated for a further 30 minutes at RT.

The unbound conjugate was removed by washing each well with 250 µL washing buffer (three repetitions as above). An enzyme-substrate (50 µL) and chromogen (50 µL) were added and incubated in the dark for another 30 minutes RT. A stop solution (100 µL) was added to each well, mixed gently by shaking the microtiter plate and the absorbance measured within 30 minutes using a microtiter plate spectrophotometer (450 nm). The absorbance of the samples is proportional to the gliadin concentration which is multiplied by a dilution factor of 500. The gluten concentration

of the samples was estimated by multiplication of the gliadin result by a factor of 2 and results were expressed as mg/kg gluten.

2.4. Chemometrics

In this study, models were generated by partial least squares regression (PLSR) using a leave one out cross-validation method. PLSR is a powerful chemometric analysis technique commonly applied in the quantification of adulteration contents in various food products (Kasemsumran et al., 2005). Multivariate calibration analysis through PLSR allows observations of linear mathematical correlation between independent variables X (spectral data) and dependent variable Y (concentration of adulterant) (Godoy et al., 2014). During the PLSR examination, the spectral dataset is compressed into orthogonal structures called latent variables (LV) or factors, and these are used to describe the maximum covariance between X (spectra) and Y (level of adulterant) variables (Xu et al., 2015).

With the seventy-two spectra collected, 70% of the dataset was assigned as calibration set and the remaining 30% for the validation set. Prior calibration modelling, raw spectra of pure and adulterated samples were corrected for noise and the effects of scatter using standard normal variate (SNV), baseline (baseline offset and linear baseline correction) and a combination of SNV+Baseline preprocessing methods (Barnes et al., 1989). Mathematical pre-treatment methods were independently examined to construct PLSR prediction models of distinct GBF biomarkers. PLSR models were initially developed using the full range (400-2498 nm) and the pre-processing method with the best performance was further studied for variable selection to optimize the

prediction model using subinterval regions 1100-2498 nm and 1200-2200 nm as proposed by Leardi and Nørgaard (2004).

Models were compared and the accuracy of the predictive PLS model was assessed by the coefficient of determination for cross-validation (R^2_{cv}) and coefficient of determination for prediction (R^2_p); root mean square error of cross-validation (RMSECV) and prediction (RMSEP); and residual predictive deviation (RPD). As a rule of thumb, an optimal predictive model results in a coefficient of determination close to one, lowest root mean square errors and must indicate good practicality by yielding RPD values of 3 and above (Ye et al., 2018).

2.5. Statistical analysis

Data of reference sample characteristics were expressed as mean ± standard deviation and were subjected to SPSS Statistics Version 20 (IBM Corp., Armonk, NY, USA). The means were compared using Independent Sample T-test at a 95% confidence interval. For spectral data analysis and development of quantitative chemometric models, the Unscrambler X (Version 10.3, Camo Process, SA., Norway) software program was employed.

3. Results and discussion

3.1. Raw flours general characteristics

The reference traits of pure flour samples are shown on Table 1. There was a highly significant ($P < 0.001$) difference in the gliadin and gluten of samples. Pure GBF samples showed absence of gliadin (-5.28 ± 0.07 mg/kg) and gluten (-10.57 ± 0.13 mg/kg). Pure wheat flour showed gliadin

and gluten contents of 43.20 ± 1.78 and 86.40 ± 3.55 mg/kg, respectively. The total crude protein composition of samples showed a highly significant ($P < 0.001$) difference with wheat flour having more proteins (11.94 ± 0.12 %) than banana flour (3.91 ± 0.15 %). Banana flour and wheat flour samples contained crude protein composition in-agreement to those reported by Lioa and Hung (2015); and Adhikari et al. (2016), respectively. This observation was therefore an indication that there was no-cross contamination between pure flours. With the obtained general characteristic results, it was attributed that indeed GBF in the current study was gluten-free whereas wheat flour was rich in gluten proteins. This gave further attributions that changes to adulterated banana flour samples would be contributed by wheat flour compositions of gliadin and gluten attributes.

Table 1: Reference characteristics of pure flours before adulteration.

Reference parameter	Flour type	
	GBF	WF
Crude protein (%)	3.91 ± 0.15^{a}	11.94 ± 0.12^{b}
Gliadin (mg/kg)	-5.28 ± 0.07^{a}	43.20 ± 1.78^{b}
Gluten (mg/kg)	-10.57 ± 0.13^{a}	86.40 ± 3.55^{b}

Samples mean ± standatd deviation. Measurements were taken in triplicate (n = 3).

Means with different letter within the same raw were significantly different ($p < 0.05$).

GBF: green banana flour; WF: wheat flour.

3.2. NIR profile of adulterated and unadulterated GBF samples

A full range of visible to near-infrared (400-2498 nm) spectra depiction of all samples is illustrated in Figure 1. There was an overlapping of sample separation at the beginning of the spectral region, however, with minimal prominent peaks (Figure 1). The differences between spectra of samples

were visibly obtained at ten positions and these were clear from 1201-2392 nm. The observed wavebands resulted from the overlapping of overtones and a combination of vibrational bands which correspond mainly to –CH deformation; -OH; -NH_2 groups stretching as well as S-H combination (Lindsay et al., 1999). Where -CH; -OH could be associated with carbohydrates, whilst -NH_2 with protein structures.

The S-H could be related to sulfuric compounds, attributed to two fractions of monomeric gliadins and polymeric glutenin single polypeptide chains (Bruun et al., 2007b); and these are the main feature characteristics of the gluten protein network that are linked by intermolecular disulfide bonds (Žilić et al. 2011; Barak et al., 2015). The absorption band at 1201 nm was assigned to the second overtone of C-H and O-H combination. The peaks at 1450; 1936 and 2012, 2106, 2224, 2320, 2392 nm were attributed to result from the first C-H; N-H and O-H stretching overtone as well as combinations of amide vibrations specific to proteins. Fundamentally amide bands II (represent N-H deformation) and III (denotes N-H and CH_2 deformation) are important in the NIR region as they show sensitivity and possible assignment of gluten structures (Bruun et al., 2007b). Therefore, wavebands identified at 2101-2392 nm were attributed to N-H, C-H combinations of quantified protein (Bruun et al., 2007a).

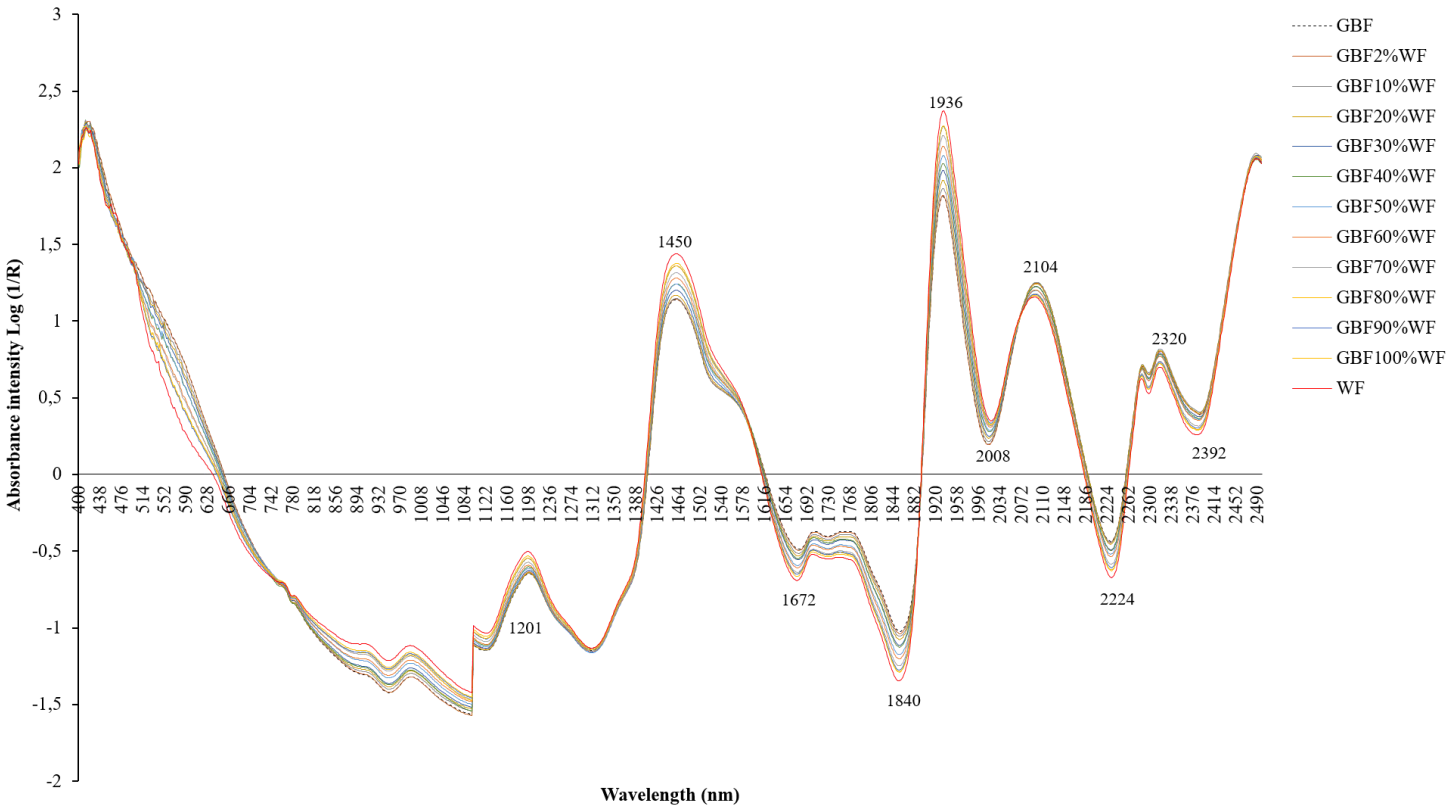

Figure 1: Typical infrared spectra with information bands derived from the NIR profile of samples obtained using SNV+Baseline transformation.

3.3. Modelling by partial least squares regression (PLSR)

Table 2 depicts the results of PLSR cross-validation and prediction models for the quantification of gluten protein adulteration. Good and reliable coefficients of determination were observed in all NIR wavelength cases. The visualization of calibration, cross-validation and prediction regression is presented in Figure 2, showing the PLS model for the experimental reference versus the predicted values. The model with the lowest root mean square error of prediction (3.9 mg/kg) was obtainable for the combined pre-processing method (SNV + Baseline). This obtained limit of detection entailed that due to the instruments' sensitiveness, the Vis-NIRS was able to respond to even low traces of gluten adulteration changes as influenced by the addition of wheat flour in GBF samples.

This predictive model also resulted in a high RPD value of 3.9 for the external test set, indicating overall accuracy and that this model would be an excellent quality control (Ye et al., 2018) in production lines to measure for gluten adulteration which will provide a satisfying banana flour product for the industry and the retail market, hence consumer protection. The model also fitted the prediction of wheat gluten very well and had the highest accuracy (R^2_p) of 0.94 (Table 2). By subjecting the model with good performance to the various spectral region it was assumed that was going to further optimize the model. However, this exercise improved model complexity by reducing the number of latent variables from 9 to 6 LV, while the predictive accuracy remained significantly satisfactory (Table 2). To the best of our knowledge, this is the first report on wheat gluten protein adulteration in green banana flour. Both wheat and banana flours are natural products characterized by complex chemical composition and different active ingredients. Given the fact that the best performing model went up to factor 9 during model development at the full

wavelength range is indicative that there may be other biochemical components (such as amino acids) that possibly influence the spectra. These components may be present in very small quantities in the adulterated sample mixtures and that correlates highly to the gluten protein, joined during modelling convey effective information to discriminate green banana flour adulteration by wheat flour concentrations. The logic inferences made from this research were drawn from Qian et al. (2008) who argued that the characterization of wheat gluten proteins may be interfered by relatively low levels of amino acids such as arginine and lysine which produces peptides with close similarity in sequence as gluten proteins; and Fontaine et al. (2002) study who concluded that NIR technique could measure amino acids concentrations by deriving them indirectly from other nitrogen-containing molecules.

Table 2: Partial least squares calibration, cross-validation and prediction models for quantifying adulteration of wheat flour gluten in adulterated unripe banana flour showing statistical parameters of spectral data using various preprocessing methods.

				Cross-validation			Test set				
Region (nm)	Pre-treatment	R^2_C	RMSEC	R^2_{CV}	RMSECV	BIAS	R^2_P	RMSEP	BIAS	RPD	LV
400-2498	RAW	0.998	0.623	0.917	4.539	-0.084	0.911	4.661	-2.235	3.300	10
	BASELINE	0.998	0.709	0.933	4.081	0.218	0.918	4.477	-2.354	3.436	10
	SNV	0.979	2.191	0.905	4.852	0.039	0.863	5.785	-2.479	2.659	5
	SNV + BSN	0.998	0.726	0.942	3.799	0.161	0.936	3.944	-2.318	3.900	9
1200-2200	SNV + BSN	0.951	3.317	0.831	6.478	0.215	0.841	6.231	-1.007	2.634	6
1100-2498	SNV + BSN	0.962	2.911	0.837	6.348	0.408	0.856	5.934	-2.170	2.766	6

R^2_C: Coefficient of determination of calibration; RMSEC: Root mean square error of calibration; R^2_{cv}: Coefficient of determination of cross-validation; RMESCV: Root mean square error or cross-validation; R^2_p: Coefficient of determination of prediction; RPD: Residual predictive deviation; LV: latent variable; SNV: Standard normal variate; SNV + BSN: a combination of standard normal variate and baseline (baseline offset and linear baseline correction).

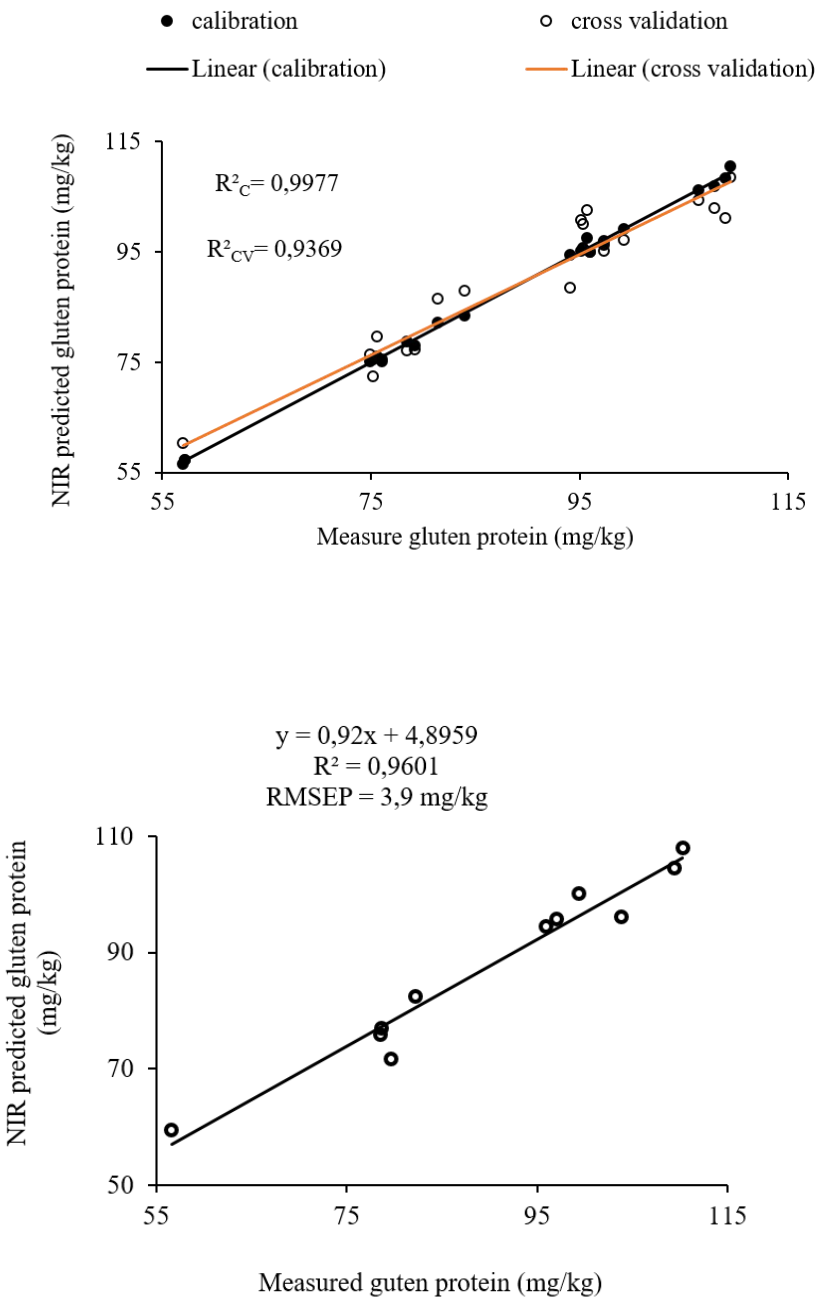

Figure 2: Scatter plots of NIR predicted versus reference gluten contents in adulterated unripe banana flour samples measured using the ELISA method.

4. Conclusion

The results from this study have demonstrated that Vis-NIRS combined with the PLSR technique can effectively detect adulteration of GBF by gluten protein from wheat. The results of this study also showed that the presence of this adulteration could be identified by ten strong and broad absorption bands observed in the NIR region. The optimal PLS predictive model showed the lowest error of prediction (3.9 mg/kg), and this was found to be below the threshold of 20 mg/kg as recommended for celiac patients. This was an indication of the spectroscopy sensitiveness and the ability of the model to detect low traces of gluten adulteration in banana flour samples. The authors believe that this approach could be a definite primary determinant of GBF processing quality that will safeguard manufacturers; protect consumers with various gluten intolerances, especially celiac disease sufferers as well as those choosing to adhere to a gluten-free diet as a lifestyle when making informed decisions during purchases. Therefore, Vis-NIRS is a very valuable, sensitive and robust non-invasive technology feasible for industrial applications to ensure the safety of GBF and related products.

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CHAPTER 7 - VIS-NIR SPECTROSCOPIC AND CHEMOMETRIC MODELS FOR DETECTING CONTAMINATION OF PREMIUM GREEN BANANA FLOUR WITH WHEAT BY QUANTIFYING RESISTANT STARCH CONTENT

Abstract

This study investigated the effect of nine different wheat adulteration levels (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% added wheat) on the concentration of resistant starch (RS) of green banana flour (GBF). The study further evaluated the potential application of visible to near infrared (Vis-NIR) spectroscopy with multivariate analysis to detect changes in the concentration of resistant starch of GBF caused thereof by wheat adulteration. Principal component analysis (PCA) and partial least squares (PLS) regression (PLSR) independently paired with 2^{nd} derivative Savitsky-Golay (with 21 smoothing gaps, 2^{nd} order polynomial); Detrend and the combination of these spectral pre-treatments were applied to compare the distribution of spectral data; model and predict the concentration of RS. A significant reduction trend in the concentration of RS of GBF samples was observed as the advancement of wheat adulteration from 38.65 ± 1.27 g/100 g (pure GBF) to as low as 5.37 ± 0.47 g/100 g (with 90% added wheat). The PCA method was able to clearly group samples and gave 93% accuracy based on RS concentration variation. The optimal PLSR models obtained after the combination 2^{nd} derivative Savitsky-Golay (with 21 smoothing gaps, 2^{nd} order polynomial) + Detrend demonstrated high accuracy with the coefficient of determination for prediction (R^2_p) of 0.97; root mean square error of prediction (RMSEP) of 2.43; residual predictive deviation (RPD) of 6.24 and a range error range (RER) of 14.27. Based on the research findings, wheat adulteration is a nutritional threat to the production and marketing of green banana flour. There is a strong potential for the tested Vis-NIR technique to rapidly monitor banana flour nutritional changes

or deteriorations caused by wheat on RS concentration. This research could provide the banana flour industry with a novel quality index to determine GBF authenticity.

Keywords: Enzyme hydrolysis, Starch crystallinity, Partial least squares regression, Quality index

1. Introduction

Powdered agricultural products are ranked the second most susceptible to adulteration after edible oils (Wielogorska et al., 2018). Green banana flour (GBF) is one of the novel and premium food products that recently has been found to be prone to intentional or unintentional adulteration. Adulteration undermines the foods' functional qualities shared with consumers' well-being. It also has a negative impact on the domestic and international opportunities of the product (Gebremariam and Brhane, 2014).

According to previous reports, the starch content of different food powders is rated on the extent of its absorption and digestibility in the small intestines (Raigond et al., 2015) as well as on the therapeutic contributions to human non-communicable diseases (Sharavathy et al., 2001). Food items such as GBF and wheat flours are judged on their nutritional importance of the starch fraction. Starch can be classified as rapidly available, slowly digestible and resistant starch (Fuentes-Zaragoza et al., 2010). Rapidly available starch is a type of starch that is digestible in the small intestines into glucose molecules within 20 minutes after a meal; whereas slowly digestible starch is the starch fraction that is converted into glucose molecules after 120 minutes after ingestion (Englyst et al., 1992; Chung and Hoover, 2009; Raigond et al., 2015).

From the viewpoint of GBF processing, resistant starch is considered an important dietary fibre and a novel quality attribute of this end-product (Fuentes-Zaragoza et al., 2011). The potent function of GBF RS to human physiology arises from the fact that it is not hydrolysed after 120 minutes of ingestion (Englyst et al., 1992; Raigond et al., 2015) in the small intestines. However, it is fermented by the colon microflora and at a later stage releases short-chain fatty acids which act as energy substrates that promote the growth of a good gut microbiome that induce minerals absorption and help inhibit the formation of colon cancer and other chronic bowel inflammatory-related illnesses such as ulcers (Mohapatra et al., 2011; Joshi and Sarangi, 2014; Dupuis et al., 2014; Ashwar et al., 2016).

In the Western regions, previous research on the applications of resistant starch includes an *in vivo* clinical study by Raban et al. (2002) where ten healthy normal weight male subjects were given for consumption meals containing no resistant starch and meals with 50 grams resistant starch content showed significantly lower concentrations of blood glucose and insulin, postprandial (after the intake of a high resistant starch meal). Moreover, Reader et al. (2002) on seven men and three women diabetic (type II) participants evaluated a variety of snack bars and showed a decrease in postprandial blood glucose and insulin levels after the consumption of snack bars composed of high resistant starch content versus low resistant starch content to no resistant starch snack bars. As for developing countries such as South Africa, a clinical study examined the effect of a high resistant starch diet on 14 male subjects who had defunctioning colostomies. The results showed that a meal rich in resistant starch increased the fermentation of short-chain fatty acids (butyrate) providing evidence that resistant starch-containing food has the potential to protect people against colorectal cancer and other bowel diseases (Ahmed et al., 2000).

Due to associated health benefits, recent studies have employed GBF in the preparation and enrichment of other food products to increase the resistant starch portion (Aparicio-Saguilan et al., 2013; Khoozani et al., 2019). Green banana flour and its resistant starch applications have encompassed the improvement of dietary fibre, water holding capacity and textural characteristics of foodstuffs such as bread (Mohamed et al., 2010); pasta (Filipović et al., 2010; Zheng et al., 2016); and confectionaries (Aparicio-Saguilán, et al. 2007; Park et al., 2010; Agama-Acevedo et al., 2012; Segundo et al., 2017).

The potential of GBF RS to provide good health benefits to consumers could be limited by the bulking with conventional cereal wheat flours mainly because they are the source of slowly and rapidly digestible starch (Hager et al., 2013). In the context of human nutrition, rapidly digestible starch is ascribed to trigger undesirable high blood glucose and insulin levels which is not good for the health of diabetes and obese individuals (Englyst et al., 1999; Hager et al., 2013). To facilitate consumers' informed decisions, novel quality properties for GBF should be protected from fraud practices which may limit consumers from obtaining full health benefits (Ashwar et al., 2016).

Traditional *in vitro* analytical methods for the determination of starches are time-consuming, involve the use of chemicals and likely to be very expensive for this evaluation at the industrial scale. A rapid method such as visible to near infrared spectroscopy (Vis/NIRS) is the key to the consistent growth and production of natural horticultural products. Along with chemometrics, Vis/NIRS is the fastest and useful technique for providing information regarding food material that has undergone minor, moderate and major composition alterations. Vis/NIRS has been successfully applied in the food industry as a quality control measure at in-

line/ and on-line process monitoring of the adulteration for various types of fruit and vegetable-derived powdered foodstuff (Fu et al., 2017; Rodriguez et al., 2019; Kiani et al., 2019).

Although in the literature most reported conventional food adulteration analysis using non-destructive methods has been based on the detection of adulteration by the measurement of weights percentages without evaluating the effects or possible changes that the adulterant could impose on the important bioactive attribute/s. An attempt made by Ding et al. (2015) on the use of reflectance near infrared (600 - 2500 nm) spectroscopy to differentiate white and purple sweet potato flours and the adulterated purple sweet potato samples showed a possibility of generating good NIR prediction models for the quantification of biochemical properties, i.e. total anthocyanins and total antioxidant activity, by radial basis function partial least squares (RBF-PLS).

However, the scientific investigation on the changes of green banana flour resistant starch (RS) due to wheat adulteration as well as the non-destructive assessment of the effects of this adulteration on this attribute has not yet been reported. Therefore, a rapid detection method and an analytical procedure to ensure the quality and safety of green banana flour resistant starch composition is necessary. This research aims to investigate the effects that wheat adulteration could cause on the RS contents of GBF and to evaluate the ability of Vis/NIRS paired with chemometrics to quantitatively predict the adulteration based on the RS concentration changes.

2. Materials and methods

2.1. Banana flour preparation

Banana fruit material was provided by the Agricultural Research Council - Tropical and Subtropical Crops (ARC-TSC). A set of 23 banana fruit cultivars (Table 1) were prepared into

flour. In a 1:1 ratio, all 23 prepared flours were blended to develop a good representative of a composite green banana flour (Ndlovu et al., 2019). Wheat flour was already prepared and obtained from a local supermarket, Mills Spar Supermarket, Pietermaritzburg, South Africa.

Adulterated banana flour samples were prepared by mixing 20 g (n = 27) banana flour samples with different proportions of wheat flour in treatment ranges of 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90%. A total of 33 batches (including n = 3 each of the pure flour samples) of green banana-wheat flour adulteration combinations were stored in polythene zipper bags at -20 °C till further use.

Table 1: Banana cultivars used for the preparation of GBF

Species	Cultivar Name	Genome Group
M. acuminata (AA) x *M. acuminata* (AA)	Chinese Cavendish	AAA
	Gros Michel	AAA
	Grand Negra	AAA
	Valery	AAA
	Williams	AAA
	D11*ǂ	AAA
	MCC*ǂ	AAA
	Calcutta 4	AAA
	Sordwana*ǂ	AAA
	IPB5-61	AAA
	Green Red	AAA
	Khuai Thong Raung	AA
M. acuminata (AA) x *M. balbisiana* (BB)	PK6*ǂ	AAAB
	Fhia-01	AAAB
	Fhia-18	AAAB
	Hinoon	AAAB
	Gold Finger	AAB
	Lady Finger	AAB
	Prata Anna	AAB
	Pome	AAB
	Foconnah	ABB
	Ducasse	ABB

*ǂ ARC-TSC selections.

Source: Perrier and Tézenas du Montcel, (1990).

2.2. Destructive (reference) analyses

2.2.1. *In vitro* measurement of resistant starch

The contents of resistant starch in the samples were estimated enzymatically using a glucose-oxidase-peroxidase (GOPOD) colorimetric assay (R-RSTAR 08/15, Megazyme International Ireland Ltd, Wicklow, Ireland) as described by McCleary (2002). In short, approximately 100 mg of samples were placed in corning culture tubes (16 x 125 mm) and treated with 4.0 mL pancreatic α-amylase and amyloglucosidase. The sample mixture was incubated for 16 hours at 37 ℃ in a shaking water bath. The samples were then treated with 4.0 mL 99% ethanol followed by centrifugation at 1500 g for 10 minutes. The centrifugation procedure was repeated twice by re-suspending pellets with 8 mL of 50% ethanol. Tubes with pellets were added with a magnetic stirrer bar and treated with 2 mL of 2 M KOH. The tubes were placed in an ice bath over a magnetic stirrer, 8 mL of 1.2 M Sodium acetate buffer (pH 3.8) were added with starring on the magnetic stirrer for approximately 20 minutes. Immediately, 0.1 mL amyloglucosidase was added, mixed well and tubes incubated for 30 minutes in a water bath at 50 °C. After incubation samples had their volume adjusted to 100 mL with distilled water and an aliquot of 15 mL of the solution centrifuged at 1500 g for 10 minutes. An aliquot of 0.1 mL was transferred onto glass test tubes, added with 3 mL of glucose oxidase peroxidase (GOPOD) reagent enzyme, and incubated for 20 minutes at 50 °C. The sample absorbance was measured using a spectrophotometer at 510 nm against a mixture of 0.1 mL of 100 mM sodium acetate buffer (pH 4.5) and 3.0 mL GOPOD reagent. The concentration of resistant starch of samples was estimated using Eq. 1 and expressed as g/ 100g of dry weight.

Furthermore, pure flour samples were initially checked and verified for possible contamination before adulteration by measurement of solubilised (non-resistant) starch (SS) and total starch (TS) contents. For the determination of solubilised starch, supernatant obtained from the 50% ethanol washing were combined with the ones of 99% suspension, with the volume adjusted to 100 mL using 100 mM sodium acetate buffer (pH 4.5). An aliquot (0.1 mL) of the solutions was added with 10 µL (amyloglucosidase and 0.1 M sodium maleate buffer (pH 6)) and incubated for 20 minutes at 50 °C. A 3 mL of glucose oxidase peroxidase (GOPOD) reagent was added and samples incubated for a further 20 minutes at 50 °C. The sample absorbance was measured using a spectrophotometer at 510 nm against a reagent blank (a mixture of 0.1 mL of 100 mM sodium acetate buffer (pH 4.5) and 3.0 mL GOPOD reagent). The absorbance data were converted by reference formula (Eq. 1) into g/100g of dry weight. The total starch content of pure flour samples was calculated as the sum of the resistant starch and solubilised (non-resistant) starch (McCleary, 2002).

$$\text{Resistant starch (RS)/ solubilised (non-resistant) starch} = \Delta E \times F/W \times 90 \quad (1)$$

Where ΔE = absorbance against reagent blank;

F = conversion factor (100 divided by the GOPOD absorbance);

W = dry weight of analysed sample

2.3. Spectra acquisition by Vis-NIRS

A laboratory bench-top monochromator NIR 6500 Systems Model XDS spectroscopy (Foss NIR Systems, Inc., Maryland, USA) was utilised to acquire spectra of pure and contaminated flours. The instrument was facilitated with a quartz halogen lamp and lead sulfur (PbS)

detector, used to measure the reflectance spectra of samples. To reduce the influence of instrumental shifts, the NIRS instrument system was calibrated with a one hundred percent white reference tile before and after every 30 minutes in between sample measurements. Spectra of pure and contaminated samples were measured in a sample cup holder with a quart glass and the flours were slightly compressed with a spatula to ensure even distribution before taking measurements. The NIR system was connected with Vision software (Vision TM, version 3.5.0.0, Tidestone Technologies Inc., KS, USA). Using a full wavelength range (400-2500 nm) spectra were collected at 2 nm interval. Each recorded sample spectrum consisted of 32 scans which were then automatically averaged and stored as log (1/R); where R represents reflected intensity.

2.4. Multivariate analysis

2.4.1. Pre-processing of the spectral dataset

Several spectral transformation methods were examined to correct for effects caused by various light scattering of spectroscopy measurements, obtain useful information and improve on the signal to noise ratio. The second derivative Savitsky-Golay log (2^{nd} order polynomial, 21 points smoothing), detrend (2^{nd} polynomial), and the combination of 2^{nd} derivative Savitsky-Golay (2^{nd} order polynomial, 21 points smoothing) + detrend were independently applied to the spectra.

2.4.2. Spectra analysis and Vis-NIRS model development

The spectral data were submitted to principal component analysis (PCA) to determine the distribution of samples according to the level of adulteration, identify outliers and determine informative wavelengths. Quantification of spectral data was performed by partial least squares

(PLS) regression (PLSR) for the construction of calibration models. The collected sixty-six spectra were randomly divided into different dataset, as calibration set (70%, n = 44) and remaining 30% (n = 22) for independent external validation set. The PLS calibration regression analysis included a duplicate of raw spectra of pure wheat and pure banana flour samples, whilst PLS validation analysis a single spectrum of each pure sample was included. The PLS calibration models were developed by the leave-one-out cross-validation method.

2.4.3. Evaluation of PLS models accuracy and performance

The PLSR models' accuracies were determined by the coefficient of determination of cross-validation (R^2_{cv}), root mean square errors of cross-validation (RMSECV), the coefficient of determination for prediction (R^2_p) and root mean square error of prediction (RMSEP). The developed calibration models' performances were evaluated by calculating the RPD and RER values. The RPD is the ratio of the standard deviation of reference data for the validation set to RMSEP and the RER is the ratio between the difference of the maximum and minimum reference values for the data in the prediction set to RMSEP (William and Norris, 2001; Yasmin et al., 2019).

2.5. Statistical analysis

Data collected were submitted to a one-way analysis of variance (ANOVA) using a Least Significant Different (LSD) post hoc test, set at $p < 0.05$ significant level. Data were expressed as mean ± standard deviation. The analyses were performed using the SPSS statistical software, Version 20 (IBM Corp., Armonk, NY, USA). Spectral data analyses were conducted using the Unscrambler X version 10.3 (CAMO Software AS, OSLO, Norway). Graphical presentations were done in Microsoft Excel.

3. Results and Discussion

3.1. Starch composition of raw green banana and wheat flours

The resistant starch (RS), solubilised (non-resistant) starch (SS) and total starch (TS) concentrations of pure GBF and WF samples were confirmed to significantly ($p < 0.05$) differed from each other (Table 2). GBF significantly exhibited a high concentration of RS (38.65 ± 1.27 g/100g) and low concentration values of SS (20.88 ± 0.47 g/ 100 g) compared to wheat flour which contained higher concentrations of RS (5.37 ± 0.33 g/100 g) and high levels of SS (64.57 ± 0.46 g/100 g). The overall starch composition of pure GBF was significantly lower (59.53 ± 1.74 g/100 g) than that of wheat flour (69.52 ± 0.78 g/100g) prior to the actual adulteration dilutions (Table 2). The concentrations of starch between pure flours were comparable to those reported by Tribess et al. (2009) and Cahyana et al., (2019), as a required standard of these flours.

Table 2: Typical variation of the proportions of RS, SS and TS composition of pure flours

Flour sample	RS (g/100 g) (d.w)	SS (g/100 g) (d.w)	TS (g/100 g) (d.w)
GBF	38.649 ± 1.268^{a}	20.881 ± 0.469^{b}	59.530 ± 1.735^{b}
WF	5.374 ± 0.329^{b}	64.574 ± 0.459^{a}	69.948 ± 0.783^{a}

GBF; green banana flour; WF; wheat flour; RS; resistant starch; SS; solubilised starch; TS; total starch; d.w; dry weight basis. Mean ± SD. Column with different letters are statistically different ($p < 0.05$; LSD post hoc test).

3.2. The resistant starch (RS) contents of green banana flour (GBF) as affected by different wheat adulteration levels

The findings of this study showed that the concentration of RS in raw GBF differed significantly ($p < 0.001$) from that of 40%WF to 90%WF adulterated samples (Figure 1). The concentration of resistant starch of pure GBF significantly decreased with advancing adulteration level from 38.649 ± 1.268 g/100 g to 23.573 ± 0.886 g/100 g (40%WF); 6.862 ± 3.651 g/100 g (50%WF); 5.939 ± 0.670 g/100 g (60%WF); 5.371 ± 0.185 g/100 g (70%WF); 5.371 ± 0.469 g/100 g (80%WF) and 5.367 ± 0.469 g/100 g (90%WF) dry weight basis. It was also observed that pure GBF resistant starch content was not significantly different from samples adulterated with 10 – 30% wheat flour (Figure 1). The concentrations of RS in banana flour samples with 40 and 50% wheat flour were significantly different ($p < 0.05$) from all adulteration levels. There were also no significant differences ($p > 0.05$) in the RS concentration of pure wheat flour and the GBF samples added with 60 – 90% wheat flour. A gradual deterioration in the RS concentration of GBF was more observable at the adulteration levels of 40 to 50%. Thereafter, the adulteration combinations of the samples containing 60 – 90% showed a consistent significant decline in the contents of RS compared to raw GBF. It was also noted that in samples containing 60 – 90% wheat flour, their RS concentrations were significantly the lowest and relatively comparable to that of raw wheat flour.

RS is a homo-polysaccharide made up of several monosaccharide units joined by linear amylose and branched amylopectin polymers of α-D glucose units bonded together by α-1,4 and α-1,6 glycosidic linkages, respectively (Ma and Boye, 2018, Egharevba, 2019). Amylopectin gives starchy foods its crystalline character that distinguishes cereal from pseudo-cereal starches. In general, green bananas are of B-type crystallinity and are not easily digested

by enzymes whereas wheat starches are of type A crystallinity and susceptible to enzyme attack (Sajilata et al., 2006).

In a study about factors affecting RS levels in food systems, Ashwar et al. (2016) reported that a decrease in RS levels of starch cointaing foods could result from any treatment causing disintegrations of the crystalline structure of the amylopectin chains. At this stage, our results demonstrated that wheat adulteration promotes a progressive deterioration of the resistant starch content, thereby negatively impacting the nutritional status of GBF. Our observations could mean that wheat starch adulteration formed associations that caused disorganisation of the granule structure and the disintegration of the crystallinity of the starch network of banana flour. High levels (40 - 90%) of wheat adulteration promoted microstructural modifications that enhanced the susceptibility of banana flour starch to hydrolysis by the enzymes, hence the decrease in RS yield. This study shows adulteration of wheat results in banana flour products of low nutritional value.

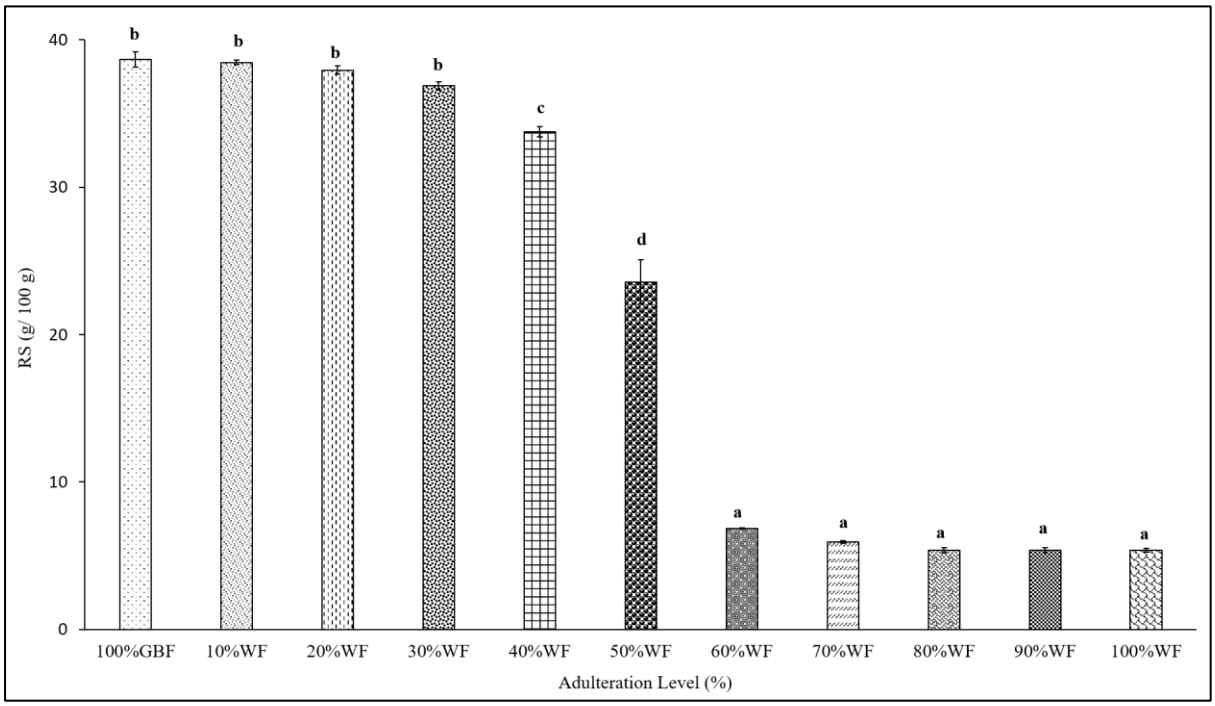

Figure 1: Changes in the resistant starch (RS) content of pure green banana flour (GBF) as affected by different levels of wheat flour (WF) adulteration. Data presented are Means ± Standard Error (SE). Vertical bars signify standard errors (SE) of the difference of means. Error bars with the same letter are not significantly different ($p < 0.05$) according to the LSD test.

3.3. Spectral analysis interpretation

The spectroscopic measurement of RS has been performed using Vis-NIR to investigate the changes in the amorphous and crystallinity (i.e. amylose and amylopectin molecular structure traits), changes in chain conformation and variability in the combinations of hydrogen bonding generate during the resistant starch formation (Ma and Boye, 2018). The important resistant starch-related absorption peaks were identified by the combination of detrending and second derivative Savitsky-Golay (with 21 smoothing gaps, 2^{nd} order polynomial) pre-treatment. Initially, the spectra of the two flours (green banana and wheat flours) were compared (Figure 2), then the variation in absorbance peaks caused by different adulteration levels was assessed (Figure 3). There were no clear bands observed in the visible region, however, most of the variation in absorption bands of GBF changed following the increase in wheat adulteration dose were clear in the NIR region (Figure 2 and 3). The broadened vibration bands suggest intermolecular differences in the hydrogen bond strength of banana flour starch with and without added wheat. This occurred at absorption bands between 1456 - 2372 nm, recognised to be the region characteristic to the analyses of starch (Subedi and Walsh, 2011). The bands at 1456 - 1556 nm can be attributed to the first overtone stretch of the hydroxyl group, causing interference on the alignment of resistant starch chains (Lv et al., 2020).

The absorption peaks between ~1964 - 2236 nm characterises the combination bands that involves the C-O stretch combination, C-C stretch vibrations (Noah et al., 1997); correspond

to the O-H stretch, O-H band combination and H-O-H deformation combination (López et al., 2017) while bands at ~2304 - 2372 nm arise from the combination of C-H bonds stretching (Aenugu et al., 2011), representing the quantified starch contents. It can therefore be said that during the adulteration, banana flour starch presumably formed cross-linkages, using hydrogen bonding, with wheat starch that interfered with the crystallinity and granular organisation of resistant starch pattern. This led to increased access to fragmentation by enzyme, hence affected banana flour RS yield.

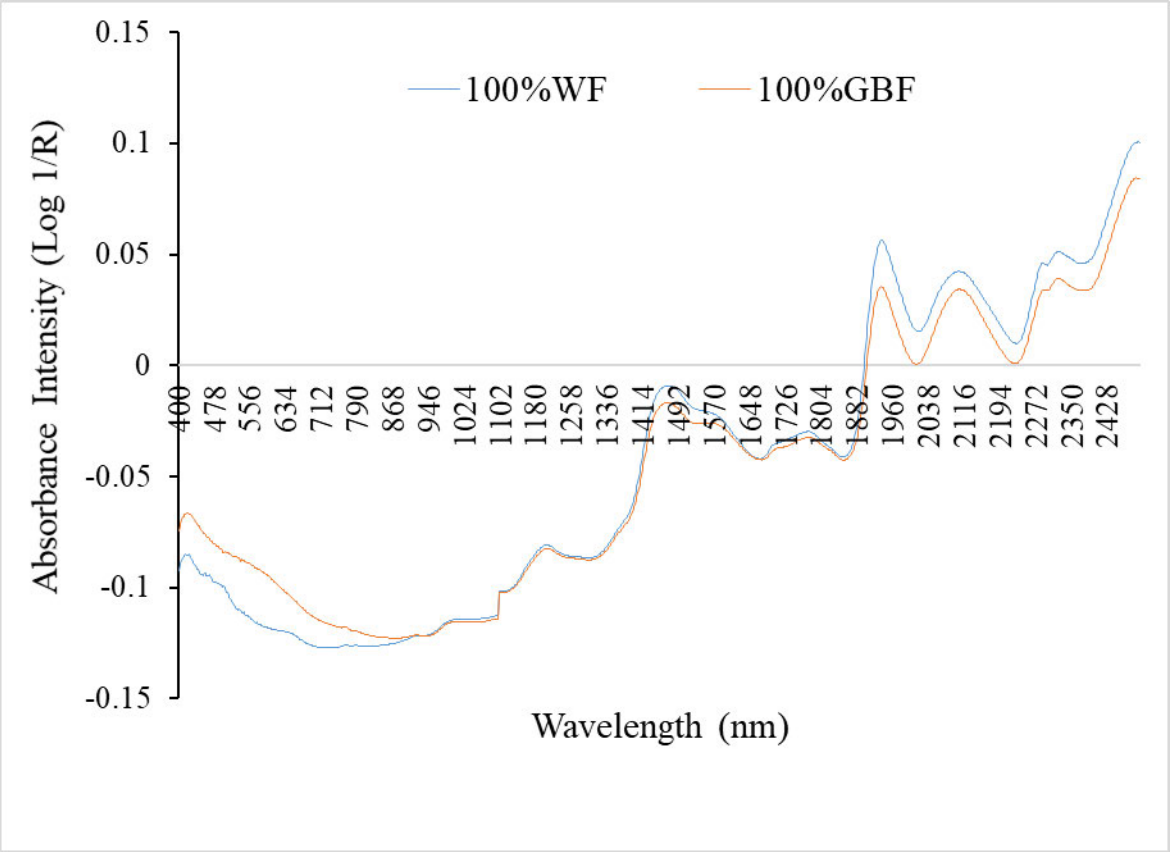

Figure 2: Spectra of unadulterated samples for the whole Vis-NIR region (400-2498 nm) after a combination of detrend and 2nd derivative Savitsky-Golay (2nd order polynomial, 21 smoothing points) pre-treatment. The blue and orange lines show spectrums for pure wheat flour (100%WF) and green banana flour (100%GBF), respectively.

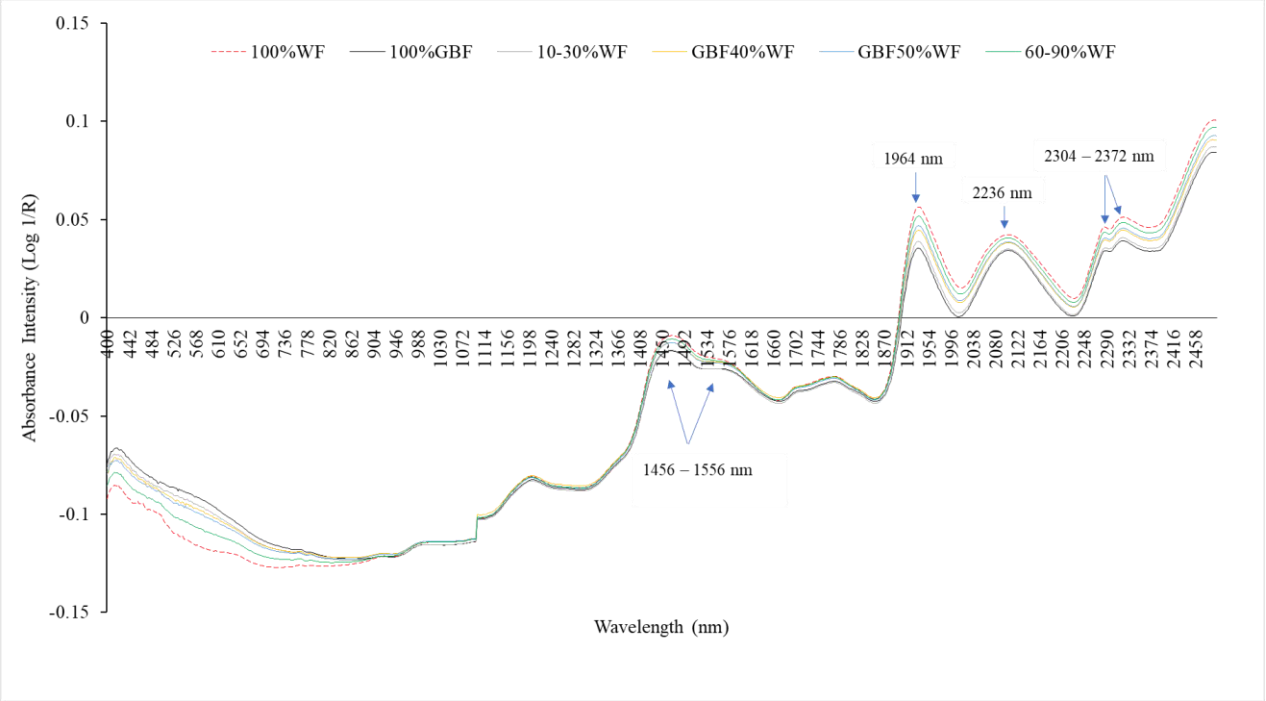

Figure 3: Typical average Vis-NIR showing differences in spectra absorption bands resulting from different wheat adulteration level.

3.4. Vis-NIR analysis using principal component analysis (PCA)

Good distributions between the samples were clearly observed in the PCA scores plot (Figure 3) on spectra transformed by the 2nd derivative Savitsky-Golay (2nd order polynomial, 21 smoothing points) and detrend (2nd order polynomial). The results of the PCA analysis showed that most of the distribution of Vis-NIR data was explained in principal component one (PC-1) and two (PC-2). Gross variability obtained between different adulteration levels amounts to 93% accuracy, where scores of PC-1 and PC-2 illustrated 88% and 5% separation between samples, respectively. The scores portrayed on PC-1 indicated a distribution of sample groupings with low adulteration level. PCA results showed that the technique can provide chat that directly indicates the quality of unripe banana flour based on the variation of the resistant starch concentration.

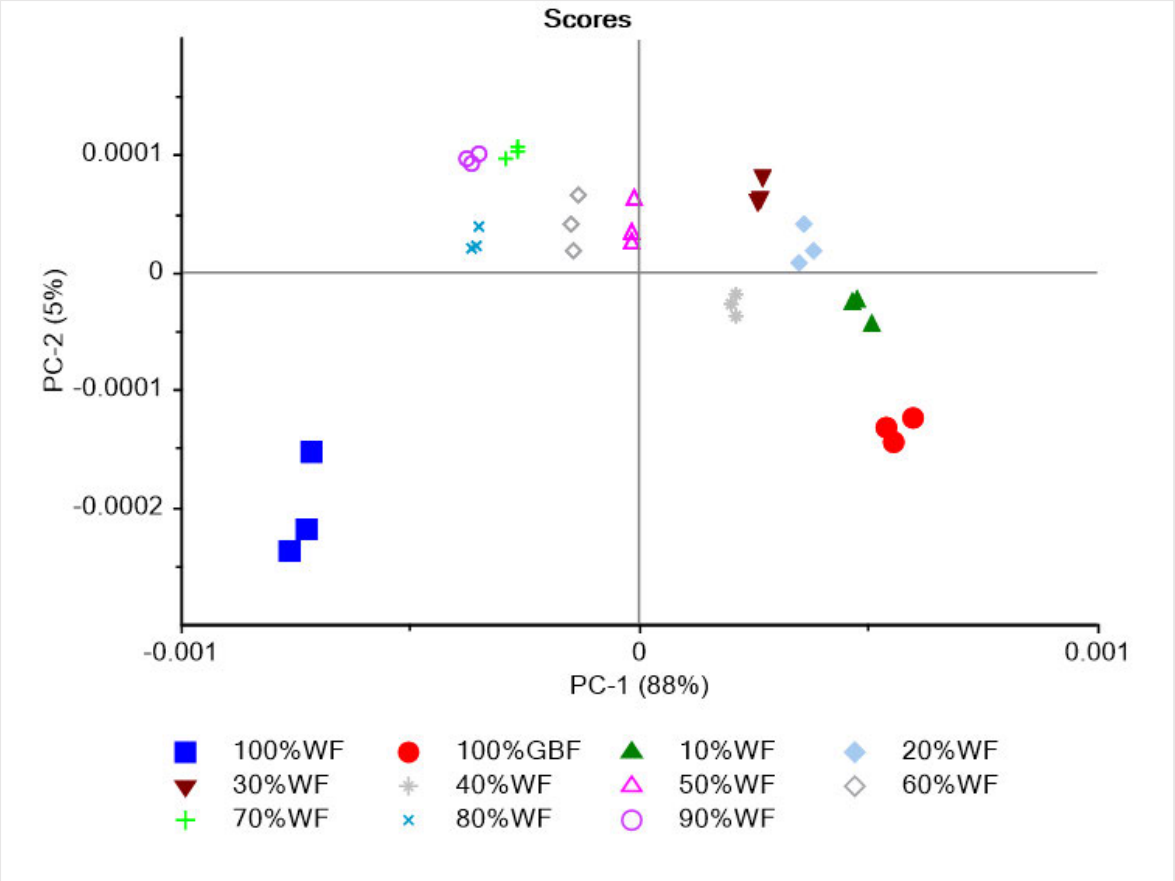

Figure 4: The PC-1 and PC-2 score plot of the combined 2nd derivative Savitsky-Golay (2nd order polynomial, 21 smoothing points) and detrend (2nd order polynomial) corrected data showing the distribution between samples with different levels of adulteration.

3.5. Quantitative prediction of RS content by partial least squares regression (PLSR) analysis

To develop Vis-NIR chemometric models, spectroscopic data with added wheat flour percentages was quantified by developing PLSR models. Shown in Table 3 are models generated to quantify and predict the wheat adulteration based on the biochemical changes of RS. The models presented a robust correlation between the actual measured values and predicted wheat concentrations. The selection of the optimal number of latent variables to use in PLSR models was based on the lowest value of the root mean square error in the cross-

validation process (Lohumi et al., 2014). The optimal models for RS were obtained with both the 2nd derivative Savitsky-Golay (21 gap smoothing points) as well as the combination of detrend and 2nd derivative Savitsky-Golay (2nd order polynomial, 21 smoothing points) pre-processed Vis-NIR spectroscopic data. These models showed accurate predictions with higher R^2_{cv} = 0.979, similar RMSECV = 2.231 and 2.229, respectively. The external validation models for these pre-processing methods have a higher R^2_p = 0.973, similar RMSEP = 2.437 and 2.433 and Bias = 0.491 and 0.490, respectively. As depicted on the plot of residual y-variance versus the number of factors (Figure 4); the models were stable at a latent variable of 5. This suggested that the number of latent variables was enough to correlate 97% of the relevant information to detect green banana powder RS adulterated with different wheat flour levels. Any addition of the latent variable would have overfitted the model (Magwaza et al., 2016).

The significant correlation between the NIR predicted and actual reference values of the prediction models were additionally assessed by checking the residual predictive deviation (RPD) as well as the range error ratio (RER). As established from previous researches, an RPD value above 3 and an RER value around or greater than 10 indicate a good and excellent model (Williams and Norris, 2001). As depicted in Table 3, both the RPD and RER results show that the models were properly developed and satisfactory for the prediction of GBF RS adulteration by wheat. Figure 5 illustrates the linear regression relationship of measure reference and NIR predicted values of the optimal cross-validation and external validation model.

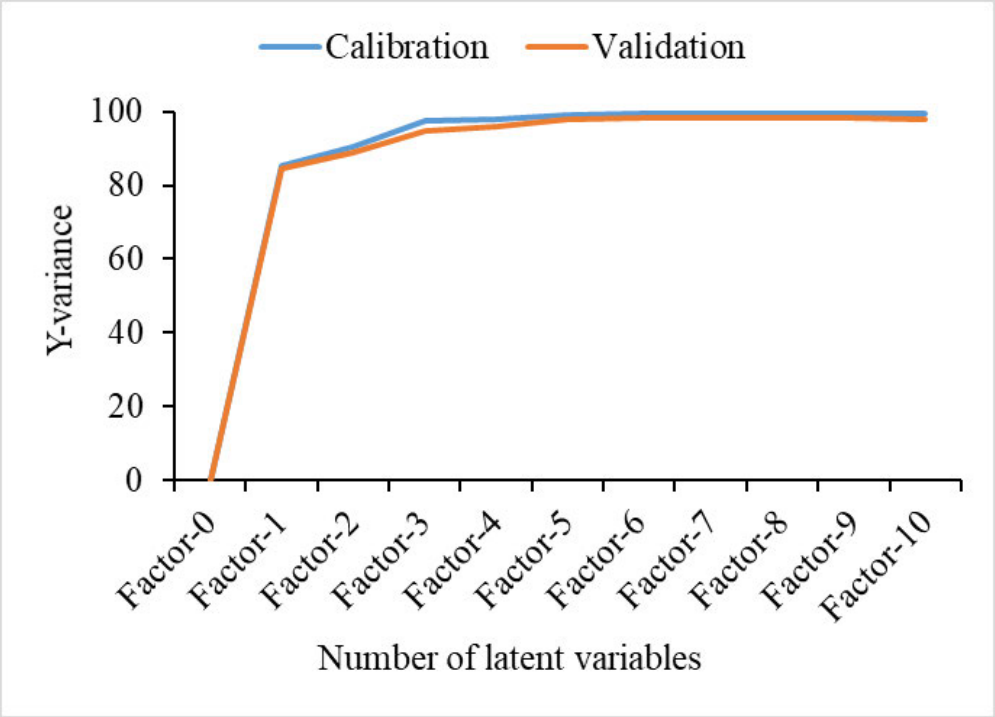

Figure 5: Residual y-variance as the function of the number of latent variables showing the optimal number of factors in the calibration model for predicting RS adulteration of GBF with wheat-based on the combination of detrend and 2nd derivative Savitsky-Golay (21 gap smoothing points, 2nd order polynomial) pre-processing method (400-2489 nm).

Table 3: Calibration and external validation models performances using full Vis-NIR (400-2498 nm) region for the prediction of banana flour RS adulteration with different wheat flour levels.

	Calibration models				Validation models						
Pretreatment	R^2_c	RMSEC	R^2_{cv}	RMSECV	R^2_p	RMSEP	Bias	RPD	RER	Slope	LV
Untreated	0.992	1.362	0.975	2.436	0.964	2.808	0.702	5.408	12.357	1.002	8
D_2 S-G_{21}	0.991	1.472	0.979	2.231	0.973	2.437	0.492	6.231	14.238	0.987	5
Detrend	0.991	1.448	0.973	2.527	0.968	2.648	0.709	5.736	13.107	0.993	7
D_2 S-G_{21}+Dt	0.991	1.474	0.979	2.229	0.973	2.433	0.490	6.243	14.265	0.987	5

R^2_c: Coefficient of determination of calibration; RMSEC: Root mean square error of calibration; R^2_{cv}: Coefficient of determination of cross-validation; RMSECV: Root mean square error or cross-validation; R^2_p: Coefficient of determination of prediction; RPD: Residual predictive deviation; RER: Range error ratio; LV: latent variable; D_2 S-G_{21}: 2^{nd} derivative Savitsky-Golay (2^{nd} order polynomial, 21 points smoothing points); Dt: Detrend.

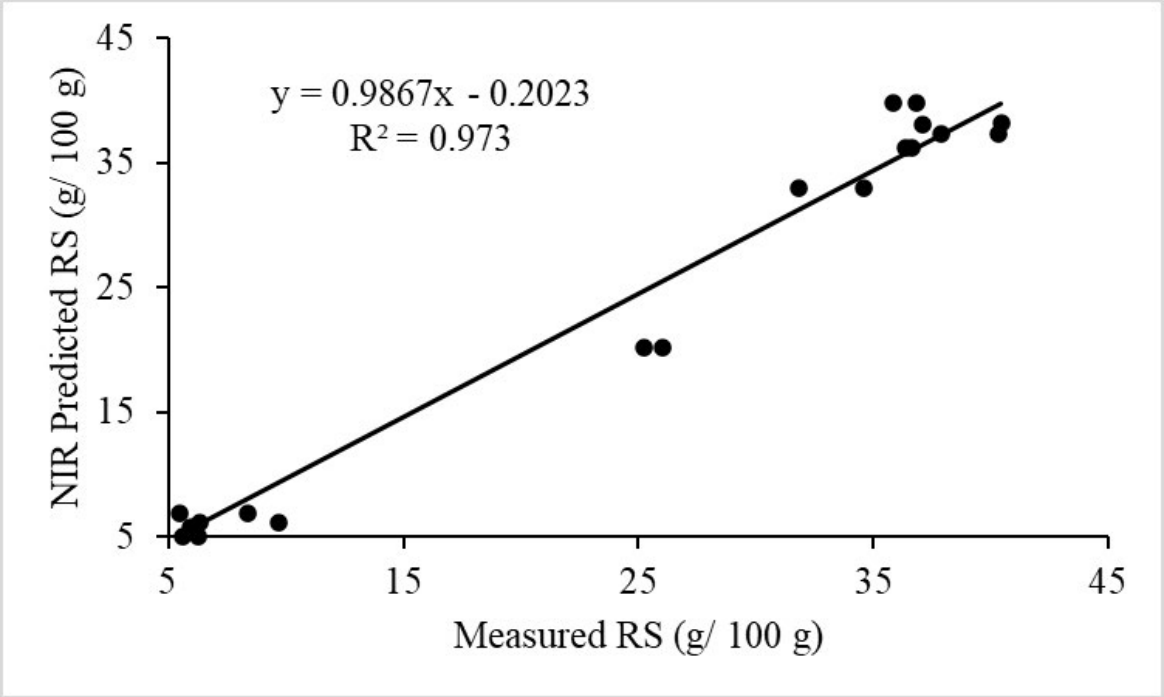

Figure 6: Scatter plot showing optimal PLS model performance to predict green banana flour adulteration with wheat-based on the resistant starch (RS).

4. Conclusion

This study has shown marked variations in the resistant starch contents of green banana flour when compared to wheat flour adulterated samples. Indeed, the advancements of wheat adulteration levels significantly reduced the resistant starch content. In other words, the potency of this novel bioactive compound is very likely to deteriorate owing to the influence caused by the adulteration of wheat. From the consumers' point of view, wheat adulteration remains a major nutritional issue and a threat to the processing and marketing of green banana flour. Vis-NIR proved to be a useful technique and explained the structural features characteristic to the variations of banana flour

starch which led to the resistant starch reduction. The Vis/NIR spectroscopic method developed in this study is fast and time saving compared to enzymatic methods. The overall accuracy of the method is effective for producers to analyse banana flour nutritional value based on the resistant starch contents. The findings of this research could enable the banana flour industry with a novel quality index to determine GBF authenticity. It is believed that the differences induced by different added wheat concentrations can be used to grade the severity of the adulteration, could allow producers to create formulations for the development of other green banana-wheat flour composite products, which might help reduce product loss induced by wheat adulteration in various food industries. Future studies should include a range of other cereals, legume or pseudo-cereal flour products, to obtain a wide variability of the impact of adulteration in banana flour RS.

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CHAPTER 8 - GENERAL DISCUSSION, CONCLUSION AND FUTURE RESEARCH RECOMMENDATIONS

1. Introduction

Unripe banana flour (UBF) is one of the vital fruit developed products appreciated for the natural gluten free characteristics and it being the source of resistant starch. In the modern society these nutritional qualities are deemed important and desirable especially for the individuals with pressing health issues such as coeliac disease, type II diabetes as well as for consumers who voluntary follow certain diets (Rodríguez-Damian et al., 2013). The processing of a high-quality unripe banana flour is therefore a priority. Regardless, there are no specific quality and safety monitoring system in place for the major producing countries which include Canada, Brazil, China, Australia, India, United States, including Africa and Southern African countries (Sardá et al., 2016a).

Unripe banana flour is one of the products susceptible to adulteration with cereal flours due to shared physical traits (Ndlovu et al., 2019). Maize and wheat flours are staples present in the daily diets of many African and Western consumers. Amongst the two, wheat flour contains a well-known allergen (gluten) of which not everybody in different parts of the world could tolerate (Scherfet al., 2016). The research study first objective was to investigate the potential of visible to near infrared (Vis-NIR) spectroscopy (Vis-NIRS) combined with multivariate analysis to develop robust prediction models that can rapidly detect and quantify different adulteration of levels of staple flours (i.e. wheat and maize flours) in unripe banana flour. the second part of the study was to explore Vis-NIRS potential to quantify quality attributes that can be used to identify unripe banana flour adulteration.

2. Destructive and rapid non-invasive methods for the detection of powdered horticultural products adulteration

The objective of Chapter 2 was to review and discuss the concept of powdered horticultural product adulteration the driving forces and its related impacts; and to discuss previous and recent researches on different techniques developed for monitoring powdered products adulteration. A literature review study showed that the adulteration of powdered food materials has been a common issue in food industries to different parts of the globe for so many years (Su an Sun., 2018). It involves the deliberate or unintentional substitution or addition of cheaper materials to high value products for the aim of increasing profits (Lohumi et al., 2015).

Practices regarding powdered products imitations are mainly influenced by greed and ease accessibility of adulterants. In addition to that, adulteration acts are sophisticated since substituting material tend to physically match the characteristics of high value products (Everstine et al., 2013; Esteki et al., 2019). There have been improvements from traditional analytical methods to non-destructive tools to monitor adulteration practices. Literature relating to existing destructive analytical methods such as chromatographic, microscopic, enzyme-linked immunological assay (ELISA), to mention a few, showed that these technologies might not be efficient for a large industrial scale. They are time consuming, expensive, need specialized sample preparation and are related to producing bias and inadequate outputs since they are usually performed on few number of samples (Ellis et al., 2012). The review study also revealed that although infrared techniques have been approved as a robust method for adulteration evaluation, its applications are not as advanced with unripe banana flour as it is with other powdered horticultural products (see Chapter 2 - Table 3). Recently, consumers demand for the assurance of good quality processed products

is high. Given that unripe banana flour is an innovative product, the lack of research and utilization of non-destructive rapid method in its processing could be a challenge to the leading producing countries as well as the food industry at large. Therefore, the use of non-invasive tools such as the Vis-NIR spectroscopy to monitor adulteration of unripe banana flour is imperative and should be thoroughly researched.

3. Portable Vis-NIR spectroscopy evaluation to detect different levels of wheat and maize flour adulteration in unripe banana flour

A handheld F-750 spectrophotometer was extensively explored for its ability to detect, classify and quantify independent adulteration levels of wheat and maize flours in unripe banana flour. In Chapter 3, the potential of the F-750 instrument (285-1020 nm) to detect wheat flour and maize flour adulteration levels from 0-80% was evaluated. Pattern recognition models developed using the 2^{nd} derivative Savitsky-Golay (19-point smoothing, 2^{nd} order polynomial)) pre-processed spectral data and principal component analysis (PCA) indicated a 95% accurate classification among adulterated and unadulterated unripe banana flour samples. Calibration and validation predictive performance of the models obtained through the 2^{nd} derivative Savitsky-Golay (19-point smoothing, 2^{nd} order polynomial) partial least squares regression (PLSR) also indicated excellent accuracy of the Vis-NIR F-750 spectroscopy.

Maize flour adulteration of unripe banana flour was studied using PLSR (Chapter 4). Various factors including temperature and wavelength range could influence the development of calibration NIR models (Campos et al., 2018). The essence of this study was to closely evaluate the feasibility of the F-750 device, by comparing how the wavelength selection range (full wavelength (447-1020 nm), visible (447-702 nm) and NIR (705-1020 nm) regions) and

temperature differences (20 and 25 °C) influences on the identification of maize flour adulterant. The optimal PLSR model showed that the Vis-NIR F-750 spectroscopy could be used as an identification tool to discriminate unripe banana flour from being adulterated with maize flour despite temperature changes, however using a full wavelength (477-1020 nm). The first derivative Savitsky-Golay (7-point smoothing, 2nd order polynomial) showed identifiable band differences between adulterated versus unadulterated banana flour. The bands noticeable were associated with changes in chemical and physical functional groups assigned to C=O, C=C, O-H, N-H and C-H, overtones and combination vibrations (Riedl et al., 2015). The results of the study showed that the Vis-NIR F-750 spectroscopy could be used as an identification tool to discriminate unripe banana flour from being adulterated with maize flour despite temperature changes. The results of PLSR predictive model for a raw full wavelength (477-1020 nm) were more superior than for the pre-processed spectra of visible (447-702 nm) and NIR (705-1020 nm) regions. This added a better understating of utilizing the instrument, another adulterant that can be detected and widened the feasibility of the spectroscopy and multivariate regression for analyzing complex adulterations for unripe banana flour.

Chapter 5 objective was to optimize the capabilities of the F-750 spectroscopy to build one global PCA classification and PLS regression models that will simultaneously predict both the adulteration of maize and wheat flours. The first derivative Savisky-Golay (2nd order polynomial, 9-smoothing gap points) transformed the Vis-NIR spectra and gave a simultaneous discrimination of pure flours and corresponding adulteration combination of adulterants. The PCA scores chart demonstrated a 94% accurate separation; and the loading plots of PC-1 and PC-2 illustrated informative and characteristic bands diversity between samples. The bands on PC-1 (515 nm, 676 nm, 742 nm to 961 nm) and PC2 (498 nm, 676 nm, 970 nm) loading were due to the stretching of

–C=O-H- and -C=C-C- of carbonyl aromatic and benzene ring compounds; and second overtone of hydrogen stretch and –N-H-, amides and amines; and third overtone stretching of –O-H- and –C-H- organic groups of starch carbohydrates (Stewart, 2004; Osborne, 2006, Coates, 2006). High prediction PLSR models also showed the use of F-750 spectroscopy poses a great potential in the food industry, since a large amount of adulterant concentrations from different botanical sources were identifiable and detected in a few seconds. This was an indication that the technology is capable of storing more information from single measurement, which will aid in rapid monitoring of unripe banana flour, ensuring better product quality security for consumers.

The overall observations of chapter 3 to 5 demonstrated that the F-750 technology combined with multivariate data analysis techniques is useful for applications to the banana flour processing industry. The feasibility of the technique is based on its robustness, on the fact that it is easy to handle, no sample preparations and reagents are required, environmentally friendly and it cost effective. The qualities of this technology point to a brighter near future for post-processing quality control as well as the management of unripe banana flour containing addition of un-declared cereal or grain flours in South Africa and abroad.

4. Identification of unripe banana flour adulteration by quantifying effective quality parameters - a benchtop spectroscopic study

It is inadequate to develop a postharvest technique for quality monitoring without having quantified that products' highly appreciated attributes. The marketing of unripe banana flour is motivated by its gluten free natural traits. Gluten is a major wheat allergen (Sharma et al., 2015; Scherf et al., 2016) and also a biological marker known to uniquely separate wheat from unripe

banana flour. In chapter 6, this study introduced a good adulteration control parameter for unripe banana flour, which has direct relationship with the nutritional quality of unripe banana flour. Wheat gluten was quantified up to an adulteration concentration level of 100%.

This was an excellent way to develop a robust predictive model for detection the authenticity of unripe banana flour and distinguishing adulteration with wheat. High significant difference ($p < 0.001$) was observed between unripe banana flour and wheat flour based on gluten protein characteristics, with our banana flour showing no traces of gluten in its natural pure state. The identification of wheat gluten adulteration, made by studying the variations of spectral bands across the spectral region (1201-2392 nm) were assigned to C-H; N-H and O-H stretching overtone as well as combinations of amide vibrations (Bruun et al.,2007a; Bruun et al, 2007b). Good and reliable gluten detection PLSR model obtained from a combination of baseline (offset and baseline linear correlation) and standard normal variate (SNV) demonstrated the power of combining preprocessing method for better predictability.

A development of a method that can measure the extent of adulteration, based on the nutritional and microstructural changes of a product is imperative. Resistant starch (RS) is a major attribute of unripe banana flour that constitutes a high potion of the starch component (Pragatiet al., 2014). The quality of unripe banana flour depends on the resistant starch composition. An in-depth study on the effects of the addition of different concentrations of wheat flour on the resistant starch concentration of unripe banana flour was investigated and reported in Chapter 7. The assessed adulteration concentrations of wheat range from 10% to 90%, at a 10% increase interval. It was observed that wheat flour adulteration has a negative effect of the nutritional quality of unripe banana flour. The results showed a significant decrease of RS contents from adulteration level of

40% to 90%; while RS content of unripe banana flour samples with 10% to 30% wheat concentration were significantly affected to a small extent. This decrease in RS contents was associated with increased susceptibility of banana flour starch to enzyme hydrolysis promoted by wheat adulteration. These observations supported the hypothesis that adulteration is a food quality problem (Esteki et al., 2019).

Further into the study, a Vis-NIR (400 - 2498 nm) spectral data which undergone transformation (2^{nd} order polynomial, 21 points smoothing points) + Detrend (2^{nd} order polynomial)) showed bands from 1456 nm - 2372 nm with possible microstructural modifications based on resistant starch of product under investigation. The principal component analysis (PCA) classification model pre-processed with the 2^{nd} order polynomial, 21 points smoothing points) + Detrend (2nd order polynomial) demonstrated a successful distribution of sample separation based on resistant starch concentration changes and achieved a 93% accuracy. Partial least square regression (PLSR) model developed with the 2^{nd} derivative Savitsky-Golay (2^{nd} order polynomial, 21 points smoothing points) + Detrend (2^{nd} order polynomial) pre-treatments predicted the changes caused by wheat adulteration with high accuracy. The stability of the prediction models support that Vis-NIR spectroscopy combined with multivariate data analysis tools can be used to discriminate nutritional changes of the product in question as a results of wheat adulteration.

5. Conclusion and Future recommendations

In conclusion, two variety of non-destructive spectroscopies showed their potential to develop models to inspect adulteration of unripe banana flour. The results of this research showed that the Vis-NIR tools combine with chemometrics are suitable non-chemical techniques for monitoring

unripe banana flour adulteration with staple flours. The F-750 quality meter is recommended for testing authenticity of unripe banana flour as it can be flexible to take measurements at varying temperature surroundings. Moreover, its portable nature makes it easy to carry around and take measurements for a large number of samples at any place.

However, adulteration research is complex, as unfair producers are always on the lookout for inferior lookalike material to use. On the other hand, the NIR spectroscopy has a shortcoming of developing product specific predictive models (Cortés et al., 2019). This means the models constructed herein are sensitive to the type of adulterants investigated (i.e., wheat and maize flours) as well as the quality parameters quantified. Our findings serve as a foundation for future research using other non-destructive methods. It is recommended that the research should be furthered and consider investigating other potential adulterants such as leguminous flours, as well as gluten free pseudo-cereal flours. Future studies should also consider the non-destructive differentiation of unripe banana flour from ripe banana flour, as these products differ in nutritional value and substitution is possible. The outputs of this study could provide unripe banana flour production firms with a fast, accurate, reliable and cost-effective method to monitor near future adulteration issues of unripe banana flour with staple flours, thereby ensuring that consumers are supplied with a good quality product.

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