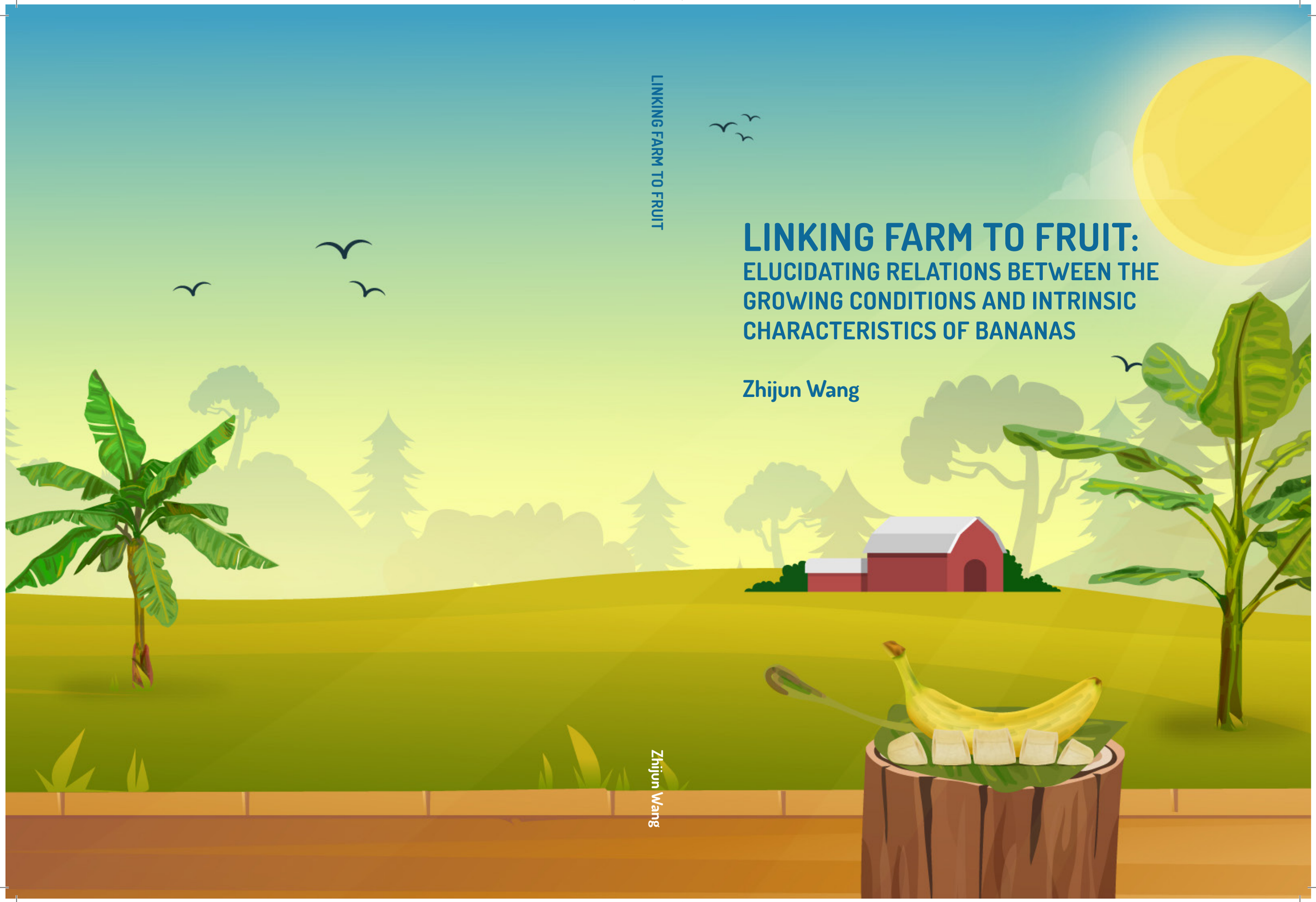
LINKING FARM TO FRUIT

Zhijun Wang

LINKING FARM TO FRUIT:
ELUCIDATING RELATIONS BETWEEN THE GROWING CONDITIONS AND INTRINSIC CHARACTERISTICS OF BANANAS

Zhijun Wang



Propositions

1. To predict the composition of the banana, its multiple growing conditions and a large sample set should be taken into account.
   (This thesis)

2. Hyperspectral imaging has the potential to link the compositional differences of bananas to their geographical origins and production systems.
   (This thesis)

3. Food fraud leads to long-term and irreversible damage to consumer confidence.

4. A deadline improves efficiency but sacrifices creativity.

5. The first step in becoming an independent scientist is to be inspired by one's intrinsic motivation.

6. The turning point of personal life and career development is often hidden in persistence.

Propositions belonging to the thesis entitled
Linking farm to fruit:
Elucidating relations between the growing conditions and intrinsic characteristics of bananas

Zhijun Wang
Wageningen, 14 June 2022

Linking farm to fruit:

Elucidating relations between the growing conditions and intrinsic characteristics of bananas

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Linking farm to fruit:
Elucidating relations between the growing conditions and intrinsic characteristics of bananas

Zhijun Wang

Thesis

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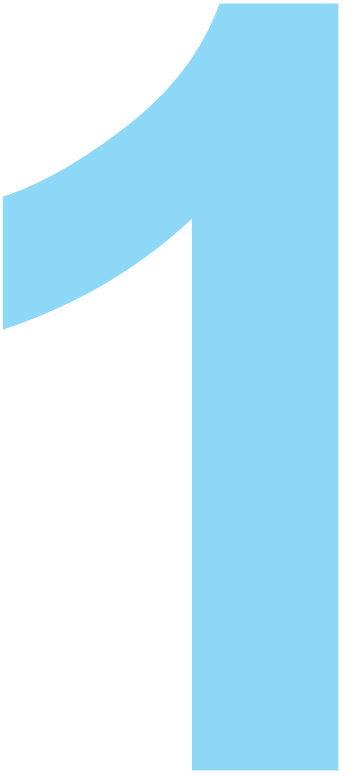
CHAPTER 1



General introduction

1.1. Bananas

The edible banana is one of most well-known fruits around the world. The most common and well-known variety is the dessert banana (*Musa acuminata* AAA) which is typically used by consumers for fresh eating and as an ingredient in desserts. It is distinguished from the cooking banana (also called Plantains) which are generally used as a staple food because of its high starch content (Price, 1995). Ripe bananas are rich in nutrients such as carbohydrates, protein, vitamins, minerals, phenolic compounds, dopamine, etc. (Pareek, 2016). The starch and glucose in banana pulp can quickly supply energy to the human body, while the rich dietary fibre in banana peels can be extracted as raw materials for other foods and industrial products (Pereira & Maraschin, 2015). Therefore, bananas are consumed directly as fresh fruits, and also used as additives in desserts, yogurt, health products, cosmetics, and various other products. In recent years, the perceived nutritional value and health benefits of bananas has contributed to its increased demand by consumers. According to Food and Agriculture Organization of the United Nations (FAO), approximately 50 billion tonnes of Cavendish bananas (subgroup of the AAA banana cultivar group) are produced globally every year (FAO, 2021a).

The main producers of banana are primarily located in tropical and subtropical regions, such as Asia, Latin America, and Africa. The favourable factors, such as suitable temperatures, irrigation, sunshine duration, and management experience with the application of fertilisers, phytosanitary measures, and pesticides contribute to increased production in these banana producing areas. The two largest banana producing countries are India and China. However, their production mostly serves the domestic market and limited product is exported. A market review of 2020 indicated that the world's leading exporting regions are in Latin America and the Caribbean. Ecuador, the largest global exporter of bananas, exported around 6.9 million tonnes in 2020 (Figure 1.1). The second largest exporter is Colombia (2.6 million tonnes), followed by Guatemala (2.4 million tonnes). The main importers of bananas are the European Union (EU), the United States of America (USA), China, the Russian Federation, and Japan (FAO, 2021b).

In recent years, the preference for organic fresh fruit of consumers is promoting the harvesting area of organic bananas. Requirements from EU 2092/91 indicated that

organic production must avoid the synthetic chemical inputs in fertilisers, pesticides, antibiotics, and food additives (Padel, Röcklinsberg & Schmid, 2009). The farmland for organic bananas must also be free from prohibited chemical inputs for at least two years before the commencing of organic production. Globally, Peru and Educator are the main organic banana producers (Dita, Garming, van den Bergh, Staver & Lescot, 2013).

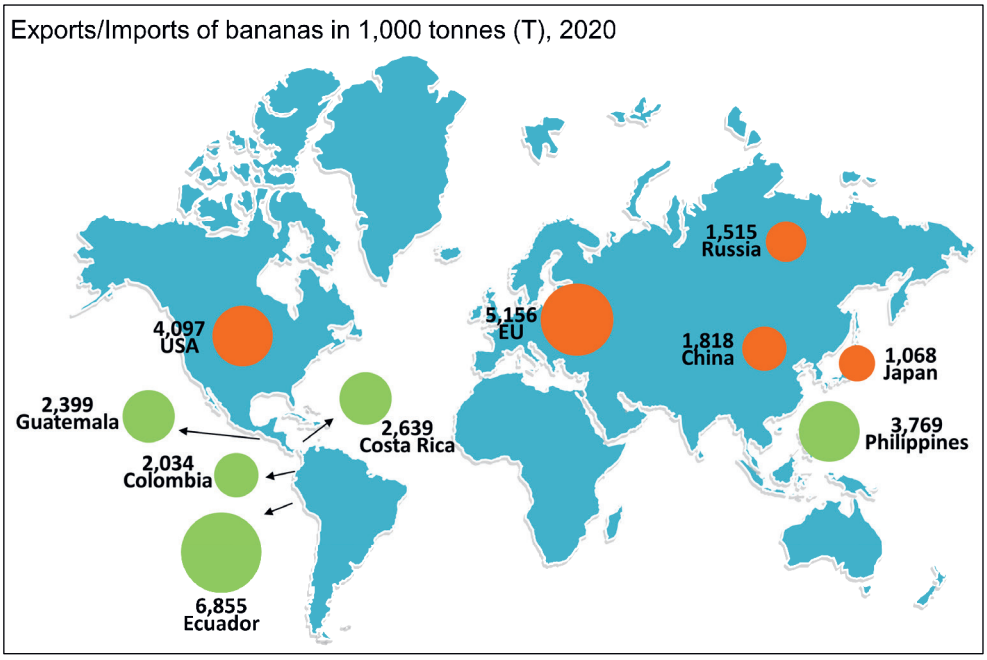


Figure 1.1. The main exporting (green) and importing (orange) countries for bananas in 2020. The map was generated from YourFreeTemplates.com within CC-BY-ND 4.0. The data used in the map was obtained from the FAO report (FAO, 2021b). United States of America (USA), European Union (EU).

1.2. The production of bananas

1.2.1. Geographical features

As typical tropical plants, bananas thrive in high temperature and humid environments, while sufficient sunshine is required in the growth period to produce high quality fruits and to obtain high yields. The optimum temperature for the banana plant is 24-32°C. This temperature promotes leaf growth, decreases the time from bud to fruit ripeness,

increases the number of buds as well as the harvest yield. Generally, there are also other environmental requirements in addition to temperature. In tropical areas with an altitude of less than 500 m, it takes 70 to 125 days from bud to harvest for bananas, while it usually takes around 110 to 250 days in subtropical areas. Temperatures below 20°C during the growth phase delay the growth and fruit development. The fruit development period increases with the increase in altitude, mainly due to insufficient accumulated temperature (Gonçalves & Kernaghan, 2014).

Bananas also require a large volume of water, for example, generally more than 100 mm of precipitation per month is necessary (Panigrahi, Thompson, Zubelzu, & Knox, 2021). Sufficient nutrient and water supply in the hot season can promote banana leaf expansion and rapid plant growth, early budding, and increase the final yield. Long-term drought usually causes banana plants to grow slowly, while the leaves turn yellow, wilt, and droop (van Asten, Fermont, & Taulya, 2011). The water shortage during the differentiation period of flower budding will also cause the number of fruits to decrease, and the banana fingers to become shorter (Carr, 2009). Under the conditions of high temperature and sufficient light, the fruit develops fast, and the fruit fingers are thick and long. However, extensive shading and strong light are not beneficial for the growth of bananas.

For the soil conditions, the commercially cultivated banana farmers normally choose clay loam or sandy loam. Bananas are ideally not planted on barren soil with a soil thickness of less than 30 cm. In that case, the barren soil must be improved with a large amount of organic fertiliser, plant branches, and leaves, pond mud, etc. before planting bananas (Gonçalves & Kernaghan, 2014). The banana farms require the groundwater level to be below 1 m, with good drainage and convenient irrigation. For soil with a high groundwater level, banana farms are prone to flooding. The optimum soil pH for bananas is around 4.5 to 7.5. This is because the fungus, *Fusarium oxysporum f. sp. cubense* (*Foc*), can multiply rapidly in acidic soils below pH 4.5, which can easily cause *Fusarium wilt* of bananas (Segura-Mena et al., 2021).

1.2.2. Farm management

The banana production mainly consists of the following activities: establishing the banana farms, fertilizations, irrigation, weed, and pest control, managing the banana

bunch, and harvest. As shown in Figure 1.2, the main differences between organic and conventional banana farms lie in fertilization management and pest control. When choosing the location of banana land, fertile, well-drained soil, plenty of sunshine, and rainfall are essential features (Meya, Ndakidemi, Mtei, Swennen, & Merckx, 2020). After satisfying these basic geographical conditions, it is necessary to carry out land preparation prior to planting bananas. At this stage, legume crops can be used as organic fertiliser to fill the soil, and then the soil is refined, deep ploughed, and levelled. Some farms also add perlite to the soil to improve drainage and ventilation as poor drainage can cause root rot. Glyphosate (Roundup®) is sprayed at a rate of 0.2 mL/m^2 before planting to keep the plantation free of weeds. Manual weeding with the help of machinery, once or twice, is also necessary (Sun et al., 2018).

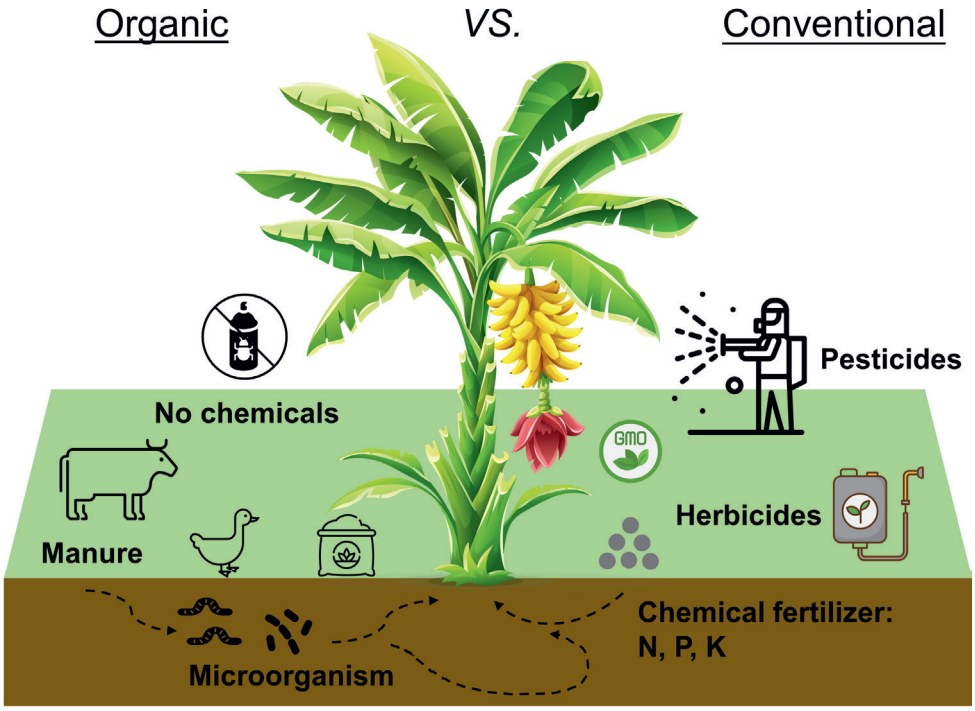


Figure 1.2. The main distinction between organic and conventional farm management of bananas. N: Nitrogen, P: Phosphorus, K: Potassium.

Plant tissue culture technology is widely used in the cultivation of banana seedlings (El-Sherif, 2018). The advantage is that many seedlings can be obtained in

a short time without pests and diseases. The development progress of the seedling could be controlled into the same line, and the use of soil can be reduced. When the seedlings have entered the farm, the periodic fertilization work begins. Traditionally, urea, phosphate, and potash fertiliser are the main chemical fertilisers used in conventional farms (Smithson et al., 2004). Organic fertilisers such as manure and compost are also applied in the production of bananas (Gonçalves & Kernaghan, 2014), which is important for improving soil fertility and reducing costs (Stoorvogel & Segura, 2018). At the same time, insecticides and herbicides are used to control pests and weeds. Banana growth requires a lot of water, and drip irrigation technology is used to improve water use efficiency on banana farms (Pawar, Dingre, & Bhoi, 2017).

The use of synthetic fertilisers, herbicides, and pesticides are strictly prohibited in organic farms (Vivek Voora, Cristina Larrea, 2020). Therefore, a lot of organic fertilisers made by hay, straw, chicken manure, seaweed as well as earthworms are used to improve the fertility of the soil of organic banana farms. At the same time, artificial weed cutting has become more important to limit the growth of weeds. In terms of preventing the manifestation of plant diseases, deep ploughing of the soil, the use of mixed herbs and lime are the main methods used (Jimenez et al., 2007; Zhang et al., 2014).

1.2.3. Banana supply chains

Bananas are mainly produced in tropical and subtropical regions such as Ecuador, Costa Rica, Colombia, the Philippines, etc., yet the main consumer market is based in countries that do not produce or have insufficient banana supply such as the EU, the USA, and China (FAO, 2021b). Resultingly, the banana supply chain network is a trade centred on exports and imports. Figure 1.3 shows the main stakeholders along the banana supply chain.

The start and end stakeholders of the banana supply chain are farms and consumers, respectively. After the harvest, the fresh, green bananas are generally packed in solid cardboard boxes with polyethylene film to avoid damage during transportation. For small holder farmers, bananas will be centralized and packaged by a professional packaging factory, while large farms are generally equipped with their own packaging lines. The packaged bananas are transported to the import destination

organized by the exporter (Svanes & Aronsson, 2013). Generally, bananas from the Philippines are mainly sold to countries such as China and Japan, while bananas from Latin America are exported to the EU and the USA in large quantities (FAO, 2020).

After long-distance shipping, the importer will transfer the bananas to the ripening factory to ripen by controlling the temperature and using a ripening agent. During this process, the starch in the banana pulp will gradually be converted into glucose and fructose, and the colour of the banana peel will also change to yellow (Borges et al., 2020; Vu, Scarlett, & Vuong, 2019). Then, the bananas will be distributed to various retailers and food service operators by wholesalers and eventually reach the consumers.

At present, multinational companies are often deeply involved in all aspects of the banana industry chain (Voora, Larrea, & Bermudez, 2020). Therefore, in some cases, different stakeholders in the supply chain could simply be different branches in one and the same multinational company. Although this control reduces the complexity of the supply chain, it could bring about a lack in transparency. This further increases the difficulty of monitoring the origin information and increases the risk of food fraud. The mistakes caused by a single company may be magnified as a huge risk affecting the entire banana supply chain. Therefore, understanding the characteristics of banana supply chains is important to assist in preventing food fraud incidents, ensure food safety and protect the economic interests of other stakeholders including consumers. The potential fraud risk deserves more accurate methods to ensure the authenticity and integrity of the final product.

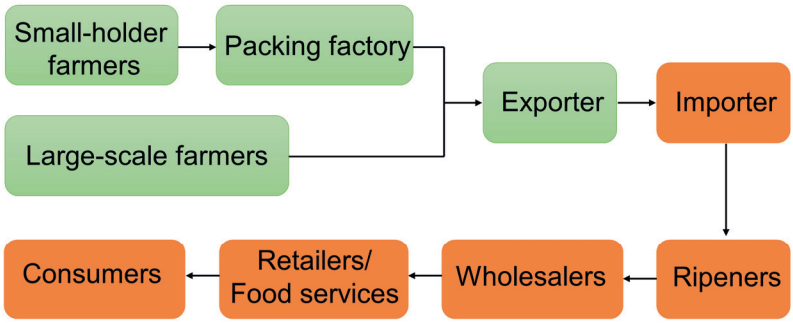


Figure 1.3. The general supply chain of bananas from farm to fork. The green boxes represent the farmer to exporter, the orange boxes represent the importer to final

consumers. (For interpretation of the references to colour in this figure, the reader is referred to the digital version of this thesis.)

1.3. The integrity of banana supply chains

According to the updated definition, food integrity is the state of being whole, entire, or undiminished or in perfect condition (FIP, 2017). Ensuring the integrity of food is essential to ensure the food safety of consumers and the economic interests of other stakeholders in the supply chain. The integrity of global foods is under constant threat from food fraud. Food fraud can be understood as an action that makes the claims of the food inconsistent with the true characteristics of the food (van Ruth, Huisman, & Luning, 2017). The vast direct negative result of food fraud is that consumers' food safety is potentially endangered. There are many potential types of food fraud such as adulteration, mislabelling, counterfeit, grey market, etc. Among them, the adulteration driven by economic interests received increased focus in recent years such as through the 2007 melamine scandal in China and the 2013 horse gate scandal in the EU (Pei et al., 2011; Brooks, Elliott, Spence, Walsh, & Dean, 2017).

Compared to other foods that are well-known to be vulnerable food fraud targets such as honey, sea food, spices, olive oil, there are not many media reports on banana frauds (van Ruth, Luning, Silvis, Yang, & Huisman, 2018). However, this does not mean that banana fruits are free from the threat of food fraud. One typical type of banana fraud is mislabelling based on geographical origin to mislead consumers and obtain higher economic benefits. Such cases mainly occur in countries with high fruit consumption such as China and India. According to the report of the FAO, India and China are among the largest banana producers in the world and these bananas are mainly supplied to their local market (FAO, 2021b). However, since the quality of bananas is closely related to the climatic conditions of the production area, the quality such as sweetness, ripeness, shape, and texture of the domestic Indian and Chinese bananas may be lower than those imported from Latin America or the Philippines. Therefore, there are fraudulent cases where local bananas are claimed to be imported bananas for sale. However, the mislabelling on geographical origin that uses domestic products to pretend to be imported products is rarely reported because the price of bananas is low, and this fraud normally will not cause public health incidents. Yet, there are some cases of abuse of illegal additives such as sulphur dioxide and formaldehyde

during the ripening process of bananas. These residual chemicals can be potentially harmful to the health of consumers (Alauddin, 2012).

Another most common banana fraud is mislabelling based on the production system, where fraudsters deliberately sell conventional bananas to consumers as organic bananas (Manning, 2016). The current banana plantation industry is very fragile to disease because commercial bananas are based on the limited genetic diversity of a single species. While organic farming provides consumers with more choices, it also faces the threat of *Fusarium wilt*, which often causes widespread wilting of all bananas in the infected area (García-Bastidas et al., 2020). To prevent such catastrophic disease, many fungicides are used on banana farms, but organic farming strictly prohibits the use of them and use organic fertilisers and microorganisms as alternative and substantiable strategies. In the soil management, applying lime ($CaCO_3$) to increase the soil pH was another effective solution to reduce *Fusarium wilt* in banana crops (Segura-Mena et al., 2021). Obviously, the difficulties associated with organic cultivation limits the production of organic bananas and resultingly drives up their prices, yet the demand for organic bananas continues to increase. Although mislabelling and adulteration in banana supply chains are not a major concern, it is still necessary to take measures to mitigate fraud, control the integrity of the banana chain, support sustainable production, and protect the rights of consumers.

1.4. Extrinsic and intrinsic characteristics for the tracing and authentication of bananas

Driven by a strong demand for high-quality and organic bananas, ensuring the integrity of the banana supply chain has become especially important. Food traceability can help verify the movement of foods products from production to consumer with paper and/or digital recording systems (Olsen & Borit, 2018). For example, information such as the production farm and organic certification are recorded in the packaging material using a machine-readable code and subsequently uploaded to a cloud database. When the code, e.g., quick response (QR) code, is scanned by consumers, the recorded information could be extracted from the cloud and shown on a mobile device (Camargo Ferraz Machado, 2019). However, full traceability in paper and digital systems does not necessarily mean the final product is authentic as an inevitable weakness for traceability systems is the falsification of original information by

production sectors. The simple example is incorrect claims on the label, such as modifying the origin and organic certification (Joenperä, Koskela, & Lundén, 2021). To combat this kind of food fraud, food authenticity testing using laboratory-based analytical methods is necessary to determine whether the final products are consistent with the claims on the packaging (Medina, Perestrelo, Silva, Pereira, & Câmara, 2019). The main difference between them is that food authenticity relies on identifying whether the composition of the food is consistent with the label using various detection methods such as classical chemical analysis, mass spectrometry, spectral analysis, and a large number of statistical calculations (Luykx & van Ruth, 2008). Food traceability focuses on the digital recording system among the food supply chains. The combination of food traceability and food authenticity can serve as a useful protective barrier for consumers.

1.4.1. Extrinsic characteristics

As described in Section 1.3, the main fraud issues concerning banana integrity are mislabelling of geographical origin and organic production. With the development of traceability technology, there are multiple verification technologies used to ensure the authenticity of the final product using extrinsic characteristics for most fruits including bananas such as a bar code, radio frequency identification (RFID), near field communication (NFC), electronic data interchange (EDI), internet of things (IoT), etc (Islam, Cullen, & Manning, 2021). Among them, QR code is one of the most common technologies used to generate a unique and non-repetitive extrinsic characteristic by binding each product circulating in the market, which has the advantages of non-copying and anti-counterfeiting (Xie & Tan, 2021). Blockchain technology is a traceability solution that has emerged in recent years. The combination of blockchain technology and QR code makes the traceability of agricultural products even more non-tamperable. It is used to track foods such as lettuce, coffee beans, scallops, and shrimps (Creydt & Fischer, 2019; Miatton & Amado, 2020). The farm needs to enter the information of the agricultural products into the QR code, such as the geographical origin, production system, production batch, production date, etc. When consumers scan anti-counterfeiting labels with barcode readers such as mobile phones, they can see the basic product information entered by the company before. According to news reports, since 2017, blockchain technology has been used to trace the origin of bananas to ensure the authenticity of the geographical origin and organic certification (Geethanjali & Muralidhara, 2021).

However, external verification technologies such as QR codes and blockchain still need to further verify the authenticity of food based on instrument inspections. Therefore, regulatory agencies in various countries have strict standards for foods with protected geographical origin such as protected designation of origin (PDO), protected geographical indication (PGI), and production system such as traditional speciality guaranteed (TSG) and organic certification (Dias & Mendes, 2018; Kahl et al., 2014). Although the development of information technology provides many reliable platforms for the authenticity of bananas, there is a lack of reliable databases based on the intrinsic markers to reflect the geographical origin and organic production of bananas.

1.4.2. Intrinsic markers and their measurement

Nowadays, multiple analytical methods are used to detect the geographical origin and production systems of food based on their intrinsic markers to provide the necessary verification information. The common analytical fingerprinting strategies use mass spectrometry, e.g., isotope ratios mass spectrometry (IR-MS), gas chromatography (GC), spectroscopy, e.g., near-infrared (NIR) spectroscopy, hyperspectral imaging (HSI), chromatography, e.g., liquid chromatography (LC), and other advanced techniques such as nuclear magnetic resonance (NMR) and real-time polymerase chain reaction (real-time PCR) (de Lima & Barbosa, 2019; Katerinopoulou, Kontogeorgos, Salmas, Patakas, & Ladavos, 2020; Uríčková & Sádecká, 2015). The combination of the above techniques has also been widely applied for complex sample analysis. For example, liquid chromatography combined with tandem mass spectrometry detection (LC–MS/MS) has been used to distinguish the geographical origin and grape variety of red wine (Jaitz et al., 2010). Besides the traditional methods, some emerging technologies were also introduced for food authentication. For example, broadband acoustic resonance dissolution spectroscopy (BARDS) could be used as a rapid tool for the classification of edible salts (Shoa, Mireei, Hemmat, Erasmus, & van Ruth, 2021). The ultrasonic pulse echo system could be used for comparation of different grades of vegetable oils (Yan, Wright, et al., 2019). However, there are no review papers about the analytical methods specially for geographical origins and production systems of bananas.

Elemental composition including the contents of the macro-, micro-, and trace elements of fruit tissues could reflect the effects of soil properties, climate

characteristics, and management methods where they grow. Inductively coupled plasma in conjunction with mass spectrometry and optical emission spectroscopy (ICP-OES) are mostly techniques used for determining the elemental compositions of food products. For instance, ICP-MS has been successfully used for the geographical identification of Chianti red wine (Bronzi et al., 2020), lemon (Ruggiero, Fontanella, Amalfitano, Beone, & Adamo, 2021), and Jackfruit (Debbarma, Manivannan, Muddarsu, Umadevi, & Upadhyay, 2021). The study about elements of lemons collected from Italy and Turkey indicted that the multi elemental profile could reflect the soils features of origin, while the harvest times seem to influence the absorption of elements (e.g., calcium) (Mottese et al., 2021). The main elemental compositions of bananas are different based on different varieties. For the Cavendish bananas, the main major elements are sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), while the minor elements are iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), and boron (B). The research conducted on the island of Tenerife indicated that geographical location (e.g., the northern and southern parts of the island) could influence the concentrations of the above-mentioned elements (Hardisson et al., 2001). The type of production system (e.g., organic, conventional) affected the elemental composition of orange juice in terms of 11 chemical elements (Turra et al., 2017). The potential reason is that the application of organic fertilization could promote a higher extractability of minerals for plants due to increased growth, gas exchange capacity of soil, and enhanced rhizosphere microbial activity (Maqueda, Herencia, Ruiz, & Hidalgo, 2011; Pogrzeba et al., 2017). Therefore, in the same way, it is expected that the elemental profiles can be used to distinguish the geographical origin and production system of bananas.

Multiple isotope ratios such as light isotopes $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{2}H/^{1}H$, and heavy isotopes $^{87}Sr/^{86}Sr$ are also useful for the identification of food authenticity. The isotope fingerprint obtained by stable isotope mass spectrometry (IR-MS) could reflect the geographical region, botanical processes, soil, and fertilization processes and fraudulent practices such as mislabelling for organic certification (Zhao et al., 2020). The principle of this technology is that stable isotopes in plants and fruits will undergo fractionation due to the influence of environmental factors such as soil, climate, and production systems. The continuous physical and biochemical reactions between plants and the growing conditions result in measurable changes in the stable isotopic

ratios. This natural difference in isotope ratios can carry information about growing conditions where the plants grow (Carter & Chesson, 2017). The stable isotopic ratios such as $\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ of wheat and its products could be used to reveal the interacted effects of geographical origin, harvest year, and processing (Wadood, Boli, & Yimin, 2019). For organic products, specifically strawberries, a previous study has shown that significantly higher $\delta^{15}N$ values were detected for organic than conventional samples (Perini, Giongo, Grisenti, Bontempo, & Camin, 2018). However, the stable isotope ratios had limited value as a single tool to differentiate plant products from different geographical origins and production systems because isotope fractionation is affected by too many factors (Inácio et al., 2020). Therefore, an increasing number of papers are reporting a combination of elemental analysis and stable isotopic ratios for food authentication such as wines (Camin et al., 2015), Maca (He et al., 2020) and durian (Zhou et al., 2021). At present, there are insufficient reports on the stable isotope ratios of bananas and their relationship with growing conditions. Therefore, these ratios will be explored in this thesis.

Volatile compounds can also be used to distinguish geographical origin and production system, because its generation and concentration changes are closely related to temperature, sunlight, rainfall, organic fertilisers, and processing methods. Gas chromatography mass spectrometry (GC-MS) is a classic analysis method for authenticity analysis of a variety of foods such as geographical origin of orange (Centonze et al., 2019), apple (Giannetti, Boccacci Mariani, Mannino, & Marini, 2017) and organic certification such as pear juice (Riu-Aumatell, Castellari, Lpez-Tamames, Galassi, & Buxaderas, 2004) and walnut oils (Kalogiouri et al., 2021). In recent years, proton-transfer-reaction mass spectrometry (PTR-MS) is also used for volatile compounds analysis of various foods and beverages such as cocoa beans from different origins (Acierno, Fasciani, Kiani, Caligiani, & van Ruth, 2019) and milks from different production systems (Liu, Koot, Hettinga, de Jong, & van Ruth, 2018). A study on passion fruit reported that organic fruit indicated threefold higher contents in ethyl 2-propenoate, ethyl hexanoate, and 2-methyl-1-propanol than conventional samples (Janzantti, Macoris, Garruti, & Monteiro, 2012). Organic cultivation could influence the synthesis of bioactive compounds from the secondary metabolism of plants such as terpenes and esters because of stressful conditions due to the lack of use of pesticides and fertilisers (Briskin, 2000). The role of geographical origin is mainly reflected in the

influence of environmental factors such as temperature, humidity, precipitation, etc. on the synthesis of volatile components. In general, esters are reported to account for 70% of the volatile profile of ripe bananas (Seymour, 1993). However, the volatile components of bananas are influenced by varieties, climatic factors, and production systems. For Cavendish bananas, it is reported that 3-methyl butyl acetate, 3-methyl butyl butanoate, 2-heptyl butanoate, 2-methyl propanol, 2-methyl propylacetate, and 3-methyl butanol constitute the main volatile compounds (Nogueira, Fernandes, & Nascimento, 2003). However, the current research on the volatile compounds of bananas is mainly focused on the role of maturity. Using the difference of volatile components to predict the geographical origin and production systems deserves more in-depth research.

Spectral characteristics have been widely applied in food authentication such as NIR spectroscopy, NMR spectroscopy, Raman spectroscopy, and HSI. The characteristic peaks in the spectrum mainly reflect the chemical composition and structural characteristics of the corresponding compositional groups, which are correlated with the location where plants grow. In terms of banana authenticity, only a few studies reported the prediction of the geographical origin of banana using mid-infrared (MIR) spectroscopy and it is believed that the degree of maturity will not affect the accuracy of the origin identification (Zhang et al., 2021). However, there are many published papers that focus on the application of spectral characteristic for food authentication in other fruits and agricultural products. In the determination of geographical origin of fruits, the classification models also played an important role. For example, least-squares support vector machine (LS-SVM) was performed to establish the discrimination model on the geographical origins of the *Goji* berries using NIR spectra (Shen et al., 2016). The combination of NIR and PLS-DA modelling allowed a fast and reliable method for classification of the strawberries from organic and conventional systems (Amodio, Ceglie, Chaudhry, Piazzolla, & Colelli, 2017). A study on coffee highlighted the potential to distinguish green coffee beans from four countries based on NIR spectroscopy (Giraudo et al., 2019). The organic production of Dutch milk was also verified by portable NIR spectroscopy combined with chemometrics (Liu, Parra, et al., 2018). Moreover, the characteristic Raman spectrum of rice was used identify rice types, varieties, and region of origin. The separation of the rice samples was due to variation in growing conditions. These differences were

linked with primary characteristic bands for the different groups detected by Raman spectroscopy such as polysaccharides, starch, protein, and lipids (Zhu et al., 2018). The possibility of HSI to trace the geographical origin of cocoa beans (Acierno et al., 2019) and automatic identification of organically farmed salmon (Xu, Riccioli, & Sun, 2017) has also been tested. However, the application of spectral characteristics in monitoring quality, geographical origin, and production system of bananas has rarely been reported.

Compared with other valuable crops, there is still a lack of knowledge on the intrinsic markers of bananas for discriminating geographical origin and production systems. Most published papers in the field of food science mainly focus on the analysis of basic components on bananas such as sugar content, polyphenol content (Singh, Singh, Kaur, & Singh, 2016), functional food research such as the nutritional evaluation of dietary fibre in banana peels (Zhang, Wang, Wang, Wang, & Huang, 2019), the food processing such as banana starch (Roman, Gomez, Hamaker, & Martinez, 2019) and the assessment of maturity stage (Surya Prabha & Satheesh Kumar, 2015). There is still a lack of systematic research on the difference between organic and conventional bananas.

1.4.3. Data analysis for banana authentication

The majority of analytical methods mentioned in the above sections produce enormous amounts of data. The terms of data analysis in food authenticity include the application of mathematical and statistical methods for computing, modelling, and visualizing data (Figure 1.4). For a small amount of featured data such as stable isotopic ratios and elemental contents, the basic statistical analysis at univariate level such as normality tests, comparing means, comparing variances, comparing proportions, and correlation analysis are widely used to show the differences of the samples' features (Nunes, Alvarenga, de Souza Sant'Ana, Santos, & Granato, 2015). For instance, the scatter plot of the mean $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope ratios of meat samples was used to show the effects of diet on the stable isotopic profiles of lamb meat (Erasmus, Muller, Butler, & Hoffman, 2018). In food authentication, however, it is common to work with analytical fingerprints and they require multivariate analysis to extract the relevant information. This kind of chemometrics is helpful to extract useful information from multidisciplinary data for supporting identification, classification, and relationship analysis.

Normally, exploratory analysis like principal component analysis (PCA) is first conducted to reduce data dimensions and display key variables. A study of olive oil showed that the PCA results could separate the extra virgin olive oil (EVOO) from lower grade olive oils and indicted the associated range of wavelengths in NIR spectroscopy (Yan, Stuijvenberg, & Ruth, 2019). Cluster analysis (CA) is another chemometric tool for explorative analysis, which aims to identify homogenous groups based on similar patterns and observations. K-means clustering, hierarchical clustering, and mean-shift clustering are popular algorithms used in many fields such as geographical origin discrimination of rice (*Oryza sativa* L.) (Lim et al., 2018) and the production method of shrimps (Ortea & Gallardo, 2015).

Moreover, the classification models are typical statistical techniques used in food authentication studies. There are various methods applied in supervised and unsupervised ways such as discriminant analysis (DA), linear discriminant analysis (LDA), partial least squares-discriminant analysis (PLS-DA), K-nearest neighbours (KNN), soft independent modelling of class analogies (SIMCA), support vector machine (SVM), etc. In these models, a confusion matrix is obtained to show calibrated and predicted classification accuracy. A classic example of the application of classification models is the geographical origin of Spanish red wines according to chromatographic profiles and PLS-DA models. Most of the samples could be classified correctly (96%) based on production regions (Serrano-Lourido, Saurina, Hernández-Cassou, & Checa, 2012). For banana research, only a few studies on the recognition of geographical and production origin of bananas have employed chemometrics so far. In a recent research study about the geographical origin of bananas, PCA was used to interpret patterns and PLS-DA was successfully used for classification models of geographical origin according to the MIR spectrum with the coefficient of determination (R^2) at 0.83 (Zhang et al., 2021).

In recent years, the Deep Learning (DL) algorithm has been widely introduced as an advanced chemometrics tool in food science. Several works have studied the application of convolutional neural networks (CNNs), feedforward neural networks (FNNs), radial basis function networks (RBFNs), and their combination with traditional chemometrics tools (e.g., SVM) in food fraud and authentication (Zhou, Zhang, Liu, Qiu, & He, 2019). In the study of ginger powder, a machine vision system and deep learning combined with CNN models could classify with 93.36% accuracy the detection

of turmeric powder fraud (Jahanbakhshi, Abbaspour-Gilandeh, Heidarbeigi, & Momeny, 2021). In addition, there is the new development of data analysis in food authentication towards data fusion and big data analysis. The advantage of data fusion is that it provides a more comprehensive and accurate screening of samples by combining outputs of multiple instrumental results rather than a single analytical method (Azcarate, Ríos-Reina, Amigo, & Goicoechea, 2021; Borràs et al., 2015). The fusion dataset comprising stable isotope ratios, elements, and fatty acids could improve the performance in geographical origin of lamb based on a PCA-LDA model (Qie, Zhang, Li, Zhao, & Zhao, 2021). Furthmore, more-scale data collection in growing conditions from farmers (e.g., geographical location, production system), hazard monitoring systems (e.g., regulatory authorities), and from consumers (e.g., consumption, performance) are also gradually introduced in food safety research (Marvin, Janssen, Bouzembrak, Hendriksen, & Staats, 2017) and are becoming promising trends driven by deep learning (Liang, Sun, Zhang, He, & Qiu, 2020; Tao, Yang, & Feng, 2020). Therefore, the combined analysis of banana composition and growing conditions is an essential step for banana authenticity research.

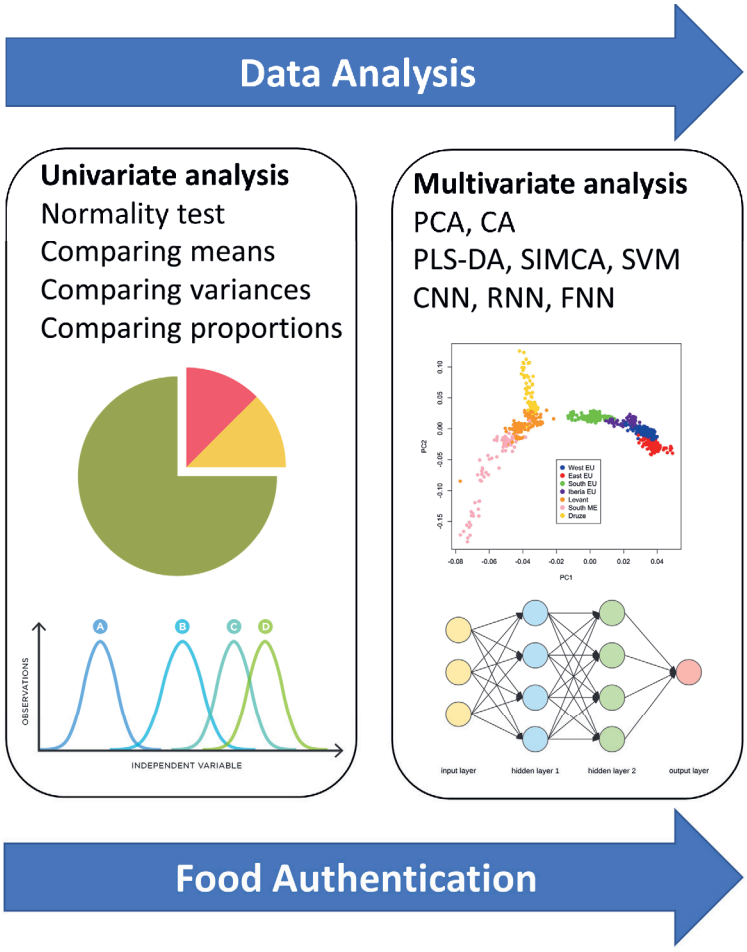


Figure 1.4. The flow chart of data analysis in food authenticity research. (Principal component analysis (PCA), Correspondence analysis (CA), Partial least-squares discriminant analysis (PLS-DA), Soft independent modelling by class analogy (SIMCA), Support-vector machine (SVM), Convolutional neural network (CNN), Recurrent neural network (RNN), Feedforward neural network (FNN))

1.5. Knowledge gap

Although the chemical compounds of bananas have been studied and the nutritional application of bananas in the food industry were also reported before, the authentication of bananas has received little attention. The consumer demand for bananas has promoted the expansion of banana production which, in combination with

its geographically long supply chain, has increased the vulnerability of banana supply chains to food integrity issues. The difference in the nutritional content of bananas provides an incentive for economically driven food fraud. High-quality bananas often come from farms with suitable climatic conditions and mature management. Organic bananas also have a higher retail price given its costly practices to meet certification requirements. Even though many instrumental analysis methods have been developed for detecting fraud in numerous products, they are not available for bananas. Furthermore, there is a lack of research on the compositional differences of bananas obtained from different growing conditions for the goal to mitigate food fraud.

The cultivation of bananas is affected by the climatic conditions of the production area. The farm location also influences soil properties. Therefore, compositional characteristics of bananas are closely related to the temperature, water, and soil properties of the geographical origin. Variation in organic bananas compared to conventional bananas are mainly due to the application of organic fertilisers and strict management systems. The geographical features (e.g., soil minerals, temperature, rainfall, altitude) and production system (e.g., organic fertilisers, irrigation) have significant effects on banana cultivation. The combined effects of geographical features and production system contribute to the compositional features of bananas. Moreover, correlation analysis based on statistics could link the growing conditions to compositional differences of bananas. This linkage is helpful to eventually derive and predict the compositional, intrinsic characteristics of bananas of growing locations from their growing conditions rather than repeating thousands of investigations on reference samples. To ensure the reliability of preventing banana fraud**,** it is necessary to understand the relationships between the growing conditions and banana compositions and provide evidence that the differences of banana compositions are the result of growing conditions such as farm locations, local temperature, altitude, and/or organic farm management. This thesis will focus on representative methods such as the elemental compositions, stable isotopic ratios, and volatile compounds, because these compounds of fruits are highly related with the growing conditions of the plants. This relationship is increasingly applied in food authentication approaches using techniques based on inductively coupled plasma-mass-spectrometry (ICP-MS), elemental analysis with isotope ratio mass spectrometry (EA-IR-MS), and gas chromatography-mass spectrometry (GC-MS), etc. (Medina, Pereira, Silva, Perestrelo,

& Câmara, 2019; Müller-Maatsch et al., 2021). In addition, spectroscopy such as near-infrared (NIR) and hyperspectral imaging (HSI) will also be introduced in detail as well to explore the characteristic spectrum influenced by the geographical origin and growing conditions.

Considering the knowledge gaps stated above, this thesis focuses on (a) the compositional characterisation of bananas from different geographical origins (climate and soil), and (b) the effects of production systems (organic and conventional system) on banana compositions. In the end, (c) the linkage of growing conditions and banana compositions is established to underpin the selection of intrinsic characteristics and for future prediction of these characteristics from local growing conditions.

1.6. Research aim

The main objectives of this thesis are to develop strategies to prevent food fraud in bananas obtained from different geographical origins and production systems. The aim is to understand the relationship between the growing conditions and banana compositions.

The detailed sub-objectives are:

(a) To understand the unique geographical features of Costa Rican bananas (micro-scale: in a single country) from different altitude, temperature, rainfall, soil, and production conditions regarding their stable isotope ratios and elemental compositions and to elucidate the linkages between growing conditions and banana compositions (**Chapter 2**).

(b) To understand the compositional differences such as stable isotopes, elements, volatile/non-volatile substances, and spectral characteristics in hyperspectral imaging of bananas from different countries in Latin America (macro-scale; across countries) and link these unique features to their growing conditions and each other (**Chapter 3-5**).

(c) To critically compare the banana features from different methods and establish the preventative models for banana fraud (**Chapter 6**).

1.7. Outline of this thesis

The overview of this thesis project is presented in Figure 1.5.

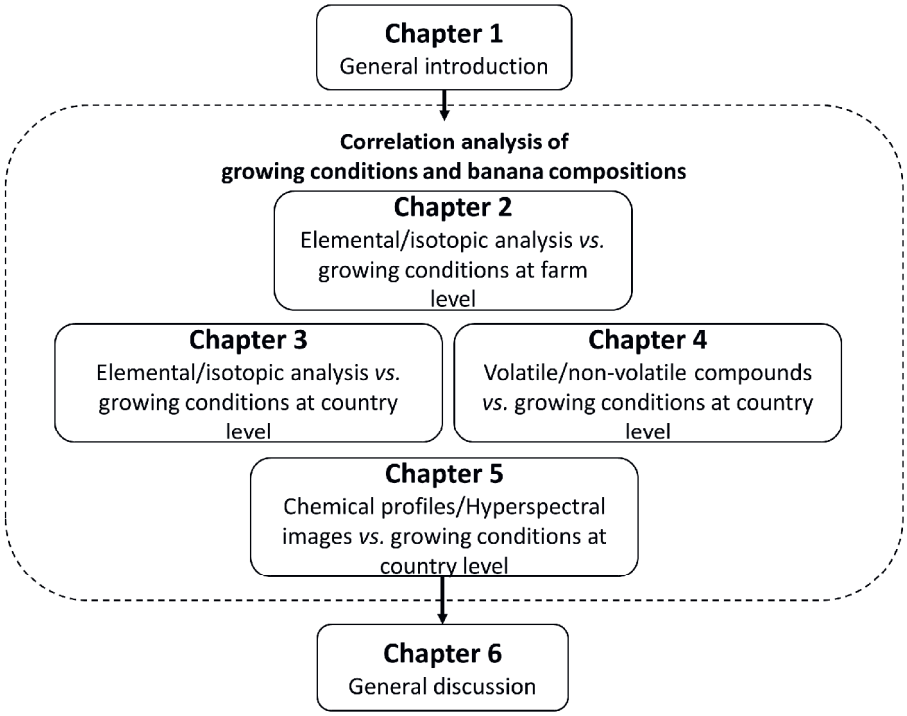


Figure 1.5. Schematic flow chart of the PhD project.

Chapter 1 provides a general introduction on the banana topic and research objectives of this thesis.

Chapter 2 explores the influence of growing conditions on the isotopic and elemental composition of bananas produced in 15 Costa Rican farms. The growing conditions are characterised in terms of climate, topography, soil conditions, and production systems. The isotopic ratios ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$) and elemental compositions of the banana pulp and peel are investigated by IR-MS and ICP-OES. Principal component analysis is performed to explore the distribution of samples from different growing conditions. Pearson correlation coefficients (r) are used to evaluate correlation between growing conditions and banana compositions in farm levels.

In **Chapter 3**, the isotopic and elemental composition of bananas collected from six different countries in Latin America are assessed, and the differences of their growing conditions like altitude, temperature, rainfall, and production system (organic or conventional cultivation) identified. Principal component analysis is conducted to reveal the separation of the farms based on geographical origin and production system. A correlation heatmap is generated to show the linkage of growing conditions and compositional attributes.

In **Chapter 4,** the composition of the bananas from the six countries in Latin America are studied in greater detail. The bananas are analysed for their volatile and non-volatile compounds by headspace solid-phase micro-extraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) and direct analysis in real time - high resolution mass spectrometry (DART-HRMS), respectively. The distribution of samples is explored by PCA according to their geographical origins and production systems. The correlation of volatile and non-volatile compounds was performed to see the interaction of banana compositions under the influences from different growing conditions.

Chapter 5 explores visual characteristics (hyperspectral images and colour values) for typifying bananas from six countries in Latin America and their relationship with general banana composition. The latter is, subsequently, related to the growing conditions of the bananas The collected visible NIR hyperspectral reflectance imaging data of the bananas are analysed by PCA to examine the bananas from different geographical origins and production systems for distinctive hyperspectral characteristics. The spectral data are subsequently correlated with the chemical compositional data (moisture, starch, dietary fibre, protein, carotene content) to comprehend the underlying causes for the distinctive characteristics. Finally, correlation analysis between the chemical profiles and growing conditions is performed to link the data to the different growing conditions of the bananas.

Finally, all the findings from **Chapter 2-5** are integrated in **Chapter 6** as the General discussion. The linkage of growing conditions and banana compositions, and banana compositions and spectral features are evaluated in this final chapter. A correlated map is generated according to the growing conditions for banana

compositions to help mitigate food fraud in banana chains. The impact, limitations, and recommendations for future research and applications are also presented.

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CHAPTER 2

Linking growing conditions to stable isotope ratios and elemental compositions of Costa Rican bananas (*Musa* spp.)

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Abstract

Traceability of agricultural produce is getting increasingly important for numerous reasons including marketing, certification, and food safety. Globally, the banana (*Musa* spp.) with its high nutritional value and easy accessibility, is a popular fruit among consumers. Bananas are produced throughout the (sub-)tropics under a wide range of environmental conditions. Environmental conditions could influence the composition of bananas. Understanding the effect of these conditions on fruit composition provides a way of increasing the fruit's traceability and linking it to its origin – a crucial aspect for the increasing global supply chain. In this research chapter, we examined the influence of growing conditions on the isotopic and elemental composition of bananas produced in 15 Costa Rican farms. A total of 88 bananas (peel and pulp) were collected from the farms and analysed for isotopic signatures ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$) and 23 kinds of elemental compositions. The growing conditions were characterised in terms of climate, topography, and soil conditions. The isotopic ratios differed significantly between groups of farms. The $\delta^{13}C$ and $\delta^{15}N$ values were mainly influenced by soil types, while rainfall and temperatures related more to the $\delta^{18}O$ values. The elemental compositions of the bananas were primarily influenced by the local rainfall and soil types, while the geographical origin could be distinguished using principal component analysis. The overall results link the growing conditions to the isotopic and elemental compositions of bananas, thereby also providing a way to trace its origin.

Key words

Banana, Elemental profiling, Geographical attribute, Stable isotopic fingerprinting

2.1. Introduction

In recent years, food with clear origin identification is favoured by consumers in view of its perceived quality characteristics (Aprile, Caputo, & Nayga Jr, 2012). This trend could also be scientifically explained by the fact that food composition can be influenced by environmental factors such as climatic and soil conditions (Granato, de Magalhães Carrapeiro, Fogliano, & van Ruth, 2016). For example, the amount of sunlight could influence the polyphenolic content in red wine (Rastija, Srečnik, & Marica-Medić-Šarić, 2009), the elemental composition of soil corresponds to the elemental profile of honey (Baroni et al., 2015), and geographical origin could change the sterol composition of goji berries (Cossignani, Blasi, Simonetti, & Montesano, 2018). Through origin identification, the traceability of a product is also improved. Traceability plays an important role in the certification or the labelling of products (e.g., organic produce) and in cases where e.g., pesticide residues or diseases are detected (Barbosa et al., 2014; Szpyrka et al., 2015).

Compounds in plant and animal products are derived from the environment, where differences in geographic features often result in different compositional characteristics of plants or animals of the same variety or breed. This opens opportunities to relate properties of agricultural produce in the marketing chain back to the growing conditions where it was produced.

A product of particular interest to study this relationship is the banana (*Musa* spp.). Globally, the banana is one of the most popular fruits. The nutritional value with its low content in fat and sugar but higher in minerals, fibre, and vitamins make the banana popular among consumers (Singh, Singh, Kaur, & Singh, 2016). In 2018, global banana exports reached a record high of 19.2 million tonnes (FAO, 2019). The European Union, the United States, and Russia are the main banana importers, while their climate is not suitable for growing bananas. Therefore, most bananas are imported from tropical and subtropical countries just like Ecuador, Colombia, Costa Rica, and the Philippines (FAO, 2019). Most export bananas are grown in Latin American and Caribbean regions due to favourable environmental conditions and the proximity to markets (Aurore, Parfait, & Fahrasmane, 2009).

Although the retail price of bananas is not that high, there is still a vast yield and consumption of bananas, as well as thousands of farmers, retailers, and consumers involved in the global banana trade. Thereby, making it a vital commodity. Banana exports are one of the key economic incomes in most of the Central American countries and Southeast Asian countries. Geographical origin identification of bananas is necessary to protect the interests of legitimate companies and ensure the income standards of farmers (van Rijn, Fort, Ruben, Koster, & Beekman, 2019). At the same time, necessary traceability technology can also protect the economic interests of consumers in banana importing countries, such as to avoid some consumers paying extra money for specific geographical origin and organic bananas (van Ruth, Luning, Silvis, Yang, & Huisman, 2018). Various papers have been published concerning the geographical origin of agricultural products, yet only a few mechanisms are well explored, especially how the growing condition influences the composition of produce. Bananas are a suitable vehicle for studying the relationship between geographic factors and food composition, given the variation in growing conditions and its importance in global trade.

To link growing conditions to the composition of edible plants, specific analytical techniques can be utilized. The analysis of stable isotopes and elemental composition is becoming pivotal as the stable isotopes and elements in plants have a close relationship with local climate and soil conditions (Potortì et al., 2018; Zhao et al., 2014). The main stable isotopes in foods, such as carbon(^{12}C), hydrogen(^{1}H), oxygen(^{16}O), and nitrogen(^{14}N), originate from nature and the growth process. The abundance or lack of these isotopes could reflect the climatic conditions, environmental conditions, and/or biological metabolism of plants or animals (Rao et al., 2017). Among these isotopes, the $\delta^{13}C$ values could be used to determine the presence of C_3, C_4, and Crassulacean acid metabolism (CAM) photosynthetic plants (Dinca, Ionete, Popescu, Costinel, & Radu, 2015). Hydrogen and oxygen are related to rainfall (Stevenson, Desrochers, & Hélie, 2015). Their composition in food is mainly dependant on the living environment, as temperature, rainfall, and irrigation all influence the distribution of the heavy (^{2}H and ^{18}O) and light (^{1}H and ^{16}O) isotopes in vapour and clouds (Dawson, Mambelli, Plamboeck, Templer, & Tu, 2002). Nitrogen in plant tissue mostly originates from the soil and chemical fertiliser. Particularly, the use of chemical fertilisers influences the $\delta^{15}N$ values (Paolini, Ziller, Laursen, Husted, & Camin, 2015). Various

studies have been performed using multiple stable isotopes to evaluate geographical effects on different products ranging from lamb meat (Erasmus, Muller, van der Rijst, & Hoffman, 2016) to milk (Scampicchio et al., 2012) to wheat (Liu et al., 2018).

The elemental composition reflects the effect of soil properties on food elements. Soil (together with fertiliser) is the main source of minerals for plant nutrition, and elemental analysis could clearly clarify the interrelationships of food and soil properties (Tyler, 2004). In a study on Romanian red wine, the elemental analysis based on inductively coupled plasma mass spectrometry (ICP-MS) showed correlations between elemental concentration in wine samples and soil samples (Geana et al., 2013). Meanwhile the $\delta^{87}Sr$ values in soil, water, and honey samples showed significant correlations as well (Baroni et al., 2015).

Even though there are many studies focusing on the composition of bananas such as carbohydrates, polyphenols, and mineral elements (Anhwange, Ugye, & Nyiaatagher, 2009; Forster, Rodríguez Rodríguez, & Díaz Romero, 2002; Pereira & Maraschin, 2015), there are limited papers linking the compositional differences to stable isotopes and elemental concentrations. In addition, the effects of climatic factors and soil condition on such differences are also limited. To the best of our knowledge, no research has been performed on the associations between growing conditions, stable isotopes, and elemental composition of Costa Rican bananas. In this paper, the results of stable isotopes and elemental profiles of bananas from 15 farms in Costa Rica are presented. The links between banana composition and growing conditions were studied. This is the first attempt to better understand the compositional differences in bananas from different geographical regions using stable isotope and multi-element analysis.

2.2. Materials and methods

2.2.1. Study area

The study focused on the Atlantic Zone of Costa Rica. Costa Rica is a major player in the global banana market exporting around 2.2 million metric tonnes of fresh fruit per year from 43,000 ha located in the Atlantic Zone of Costa Rica (FAO, 2019). Although the area is entirely located in perhumid tropics, major differences in growing conditions still occur (Segura et al., 2015; Stoorvogel & Eppink, 1995). Climatic differences occur

with for example, annual rainfall ranging from 2,300 up to 4,300 mm/year. Soils differ considerably and include young, coarse-textured, well-structured, volcanic ash soils, to well developed, fine-textured clayey soils developed in calcareous deposits. Although productivity is high throughout the region, differences occur ranging from 40 MT/ha up to 50 MT/ha.

2.2.2. Sample collection and pre-treatment

In the study area (Section 2.2.1), 15 farms were selected as the sampling sites. The geographical locations of the farms are shown in Figure 2.1. All farms produced the same banana variety, namely *Cavendish Williams*, which is one of the most widely grown cultivars in commercial farms around the world. Bananas were collected from each farm in June 2014. Five to six banana bunches were randomly selected from each farm, and bananas were then picked from the top of each bunch. The selected bananas were kept in pre-cleaned polyethylene bags and transported to Wageningen University & Research during their green or unripe stage. The bananas were then freeze dried once they were ripe. A total of 88 bananas were separated into peel and pulp, respectively. All the banana samples were lyophilized at -40°C for 3 days, and then, the peel and pulp samples were pulverized into a fine powder before the stable isotope and elemental analysis. For the development of fertilization strategies, soil conditions are constantly monitored on farms with annual soil sampling of the topsoil. Soil data from the regular soil monitoring on the farms were extracted from the large database. The soil data of 2014 were selected for analysis as it was the same year in which the banana samples were collected. Exchangeable Al, Ca, Mg, K, P, Zn, Cu, Fe, and Mn of soil samples were analysed in the research division of the National Banana Corporation (Corbana) in Costa Rica using a Mehlich III extraction (Mehlich, 1984). The details of the samples collected are shown in Table 2.1.

General data on the growing conditions were derived from Google Earth (altitude, distance to the sea), regional GIS databases (general soil type, soil texture, andic properties) (Stoorvogel & Eppink, 1995), and field observations (Wesselink, 2016).

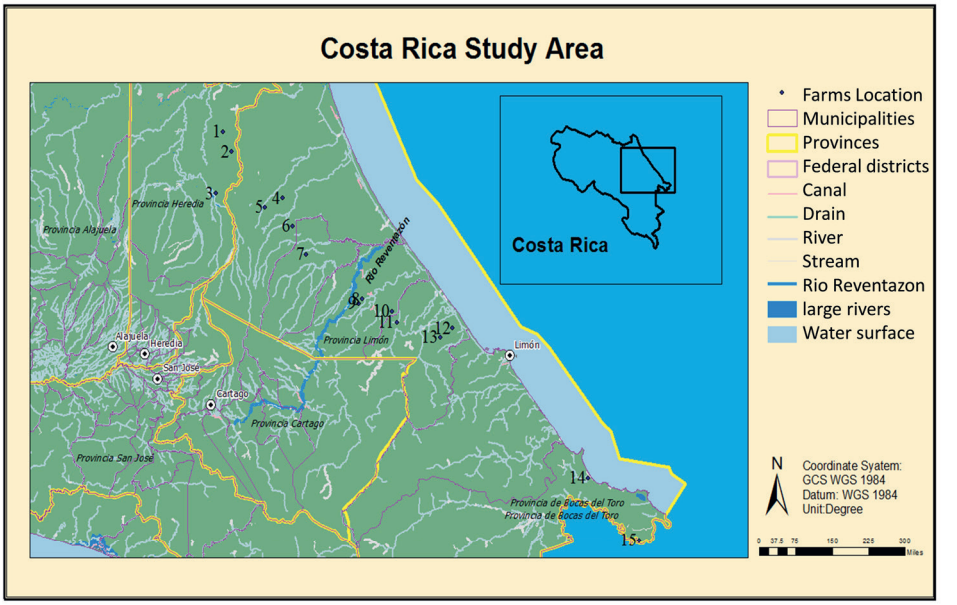


Figure 2.1. Map indicating the farms from which bananas were sampled in Costa Rica.

Table 2.1. Layout of the number of banana and soil samples collected for the stable isotope and elemental analyses from the 15 different farms in Costa Rica.

Farm	Pulp for isotope analysis[#]	Pulp for elemental analysis	Peel for elemental analysis	Soil for elemental analysis
1	6	6	6	nc
2	5	6	6	nc
3	6	6	6	12
4	6	8	8	11
5	6	6	6	36
6	6	6	6	36
7	5	6	6	49
8	6	6	6	nc
9	6	6	6	68
10	6	6	6	47
11	6	6	6	31
12	6	6	6	25
13	6	6	6	49
14	6	6	6	15
15	6	6	6	nc

[#] Only the banana samples from top position of the bunch were used for isotope analysis; (nc) Not collected due to weather problems.

2.2.3. Stable isotope determination

The stable carbon, nitrogen, and oxygen isotope ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$, and $^{18}O/^{16}O$, respectively) of banana pulp powder were measured using a stable isotope ratio mass spectrometer (IsoPrime100, Isoprime Company, Stockport, UK) combined with an elemental analyser (Vario PYRO cube, Elementar, Germany) (Liu et al., 2018). For the simultaneous determination of the $\delta^{13}C$ and $\delta^{15}N$ values, the dry samples were weighed 6.5 mg into tin capsules (5 x 9 mm, Santis Analytical AG, Switzerland). The encapsulated samples were first combusted at 1020ºC in an oxidation tube. After that, NO_2 was reduced to N_2 with copper wires at 600ºC in a reduction tube, the CO_2 was diluted by a dilutor at the same time. Ultimately, CO_2, and N_2 were introduced into the isotopic mass spectrometer by a continuous flow interface (ConFlo III) (Thermo Fisher Scientific, Bremen, Germany). For the quality control, every 12 samples were interspersed with an international atomic energy agency (IAEA) standard sample Caffeine (IAEA-600) for calibration. The stable isotope ratios ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) are described in the delta (δ) notation, and calculated against the Vienna PeeDee Belemnite (V-PDB) and nitrogen air (internal standards) using the following formula:

$$\delta\ (‰) = (R_{sample}/R_{standard} - 1) \times 1000, \quad (1)$$

where δ (‰) refers to the values of $\delta^{13}C$ or $\delta^{15}N$ and R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Standard deviations of repeated measurements of in-house standards for both carbon and nitrogen were 0.2‰. All the stable isotope ratios of $^{13}C/^{12}C$, $^{15}N/^{14}N$ were corrected using V-PDB for the $\delta^{13}C$ values and air for the $\delta^{15}N$ values.

For the determination of the $\delta^{18}O$ values, powdered pulp samples were weighed into a silver boat to an accuracy of 1 mg and were balanced for 72 h before being combusted individually in the elemental analyser. The samples were pyrolyzed in a cracking tube at 1450 ºC to form CO and H_2. Ultimately, all the gas was introduced into the isotopic mass spectrometer for determination. In the analysis sequence, every 12 samples were interspersed with a standard sample for calibration. The calibration standard for stable oxygen isotope was IAEA-601. The stable oxygen ratios were expressed as the conventional delta (δ) notation according to the following general formula (1), where δ (‰) refers to the $\delta^{18}O$ value, and R is the ratio of $^{18}O/^{16}O$. The

analytical precision for oxygen was 0.4‰. The isotope ratio of $^{18}O/^{16}O$ was correspond to Vienna Standard Mean Ocean Water (V-SMOW) as the international reference.

2.2.4. Determination of elemental composition

The 23 kinds of elemental compositions of bananas were determined using inductively coupled plasma - optical emission spectrometry (ICP-OES) at the Geolab of Utrecht University in the Netherlands (Gonzálvez, Armenta, & de la Guardia, 2011). For the analysis (performed in duplicate per sample), 125 mg of each sample was introduced into the Teflon container together with 6 mL of Aqua Regia for pre-treatment. All the containers were closed and placed overnight in a heating block at 90ºC. The next day, the containers were placed back into the heating block at 140ºC to boil down the Aqua Regia. As soon as the consistency had become gel-like (approximately 60 min), 25 mL of a 5% HNO_3 solution was added, and the containers were placed back in the heating block for 2 hours at 90ºC. All containers were left to cool after heating, whereafter the containers were weighed to determine the final dilution. After that, the sample measurements were carried out using a Spectro Arcos ICP-OES analyser (Spectro Analytical Instruments GmbH, Germany) with radial argon plasma as ion source and a Paschen-Runge spectrometer with 22 CCD detectors.

2.2.5. Statistical analysis

The stable isotope ratios and elemental data were first tested for normal distribution using the Shapiro-Wilk test (Shapiro & Wilk, 1965). After confirmation of normality, a one-way analysis of variance (ANOVA) was performed for the isotope and elemental data. The significance level was determined using the Duncan's new multiple range test and a 5% probability level was considered as significant (Duncan, 1955). Scatter plots were created to show the differences of stable isotope ratios from different regions. To further visualize grouping of the banana samples, principal component analysis (PCA) was performed using various elemental data. In our research, a series of growing conditions were collected to explore their relation with stable isotope ratios and elemental compositions in bananas. Pearson correlation coefficients (R) were used to evaluate correlation between growing conditions and banana compositions (Snedecor & Cochran, 1980). All the statistical analyses were performed using SAS

9.4 (SAS Institute Inc., Cary, NC, USA) and R 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria).

2.3. Results and discussion

2.3.1. Growing conditions at banana farms

The variation in growing conditions in the study area is summarized in Table 2.2. An important distinction that is commonly being used for management recommendation is the separation by the *Reventazón* river that divides the area. North of the *Reventazón* (with Farms 1-7), soils are predominantly of volcanic origin whereas to the South of the *Reventazón* (with Farms 8-15), soils are non-volcanic and partly developed in calcalic deposits. These differences clearly result in differences in soil conditions (Toohey, Boll, Brooks, & Jones, 2018).

The rainfall data differed among the different farms based on the microclimate caused by the elevation and the geography of the particular region in the Atlantic Zone. Table 2.2. shows the annual precipitation among the 15 different farms. All these farms could be separated into high rainfall and low rainfall regions based on variation in rainfall level. The farms (Farms 1, 2, 3, 4, 5, 6, 7, and 11) receiving 3500-4000 mm/year were located in the high rainfall region. Whereas the other farms (Farms 8, 9, 10, 12, and 13) were located in the low rainfall region with 3000~3500 mm/year. The altitude (height above sea level) and the distance from the sea of each farm were also collected and are shown in Table 2.2. Farm 1 was the farthest (38.1 km) from the ocean. Conversely, Farm 14 was the closest to the sea, being only 2.4 km away. As for altitude, the northern farms were located higher above sea level than the southern farms. The highest altitude (42 m) was observed for Farm 3 and 5, whereas the lowest was 8 m for Farm 13 and 14 (Table 2.2).

The soil features such as fertile, drained condition, texture class, and andic properties of the 15 farms soil are also summarized in Table 2.2. The soil used for banana cultivation were divided into four types: fertile well-drained (FWD), fertile poorly-drained (FPD), infertile well-drained (IWD), and peat. Normally, a farm has multiple soil types, so the soil used in farms were described by percentage of the corresponding soil type. Taking Farm 1 as an example, its soil consisted of 48% FWD, 41% FPD, 9% IWD, and 1% peat. According to the growth requirements for bananas,

fertile and well-drained soil types are better options for improving the quality and yield of bananas, therefore no farm has most of its area in the infertile or peat stratum (Zake, Bwamiki, & Nkwiine, 2000). The major soil stratum was FWD soil including Farms 5, 7, 8, 9, and 11. Another type was the FPD soil stratum, which was mainly found for Farm 10. Among them, Farms 7 and 8 had 100% FWD soil, and Farm 10 had 88% FPD soil. The other farms had a similar proportion of the above-mentioned two soil types.

The proportion of the relative areas were calculated and are shown in Table 2.2. Costa Rica has a long history for banana planting and exporting, so farmers will try to find the most suitable soil for bananas. The major texture classes are sandy loam and clay loam stratum, which could maintain adequate water retention beneficial to plant growth. Among all the farms, Farm 13 had the highest proportion (100%) of clay loam, followed by Farm 12 with 88%. For the sandy loam, there are four farms within most of the soil were sandy loam including Farm 5, 8, 9, and 11. Regarding the other farms, they had similar proportions of soil texture in sandy and clay loam. Soil texture condition are also considerable index for the growth of bananas, which could influence the absorption of water, utilization of fertiliser, and root respiration (Dexter, 2004). It refers to the relative proportion of particles with various sizes, such as sand, silt, and clay in the soil (Kilmer, 2018). Texture could indicate the air and water holding ability of the soil, even the rate at which water can enter and move through soil. Appropriate soil properties not only ensure the optimum moisture level required for banana growth, but also allow drainage water to be removed as quickly as possible to protect the banana roots.

Table 2.2 also shows the andic properties of soil collected from the farms. The andic properties reflected the result of moderate weathering of mainly pyroclastic deposits. The andic soil type was mostly found on Farm 5, with 75% andic proportion. For the other farms, Farms 8, 6, 10, had 100%, 92%, and 84% partly andic soil, respectively. Farms 11, 12, and 13 had non-andic soil. The rest of the farms had both andic and non-andic soil. According to the andic properties, the non-andic farms were clearly distinguished from all farms by the *Reventazón* river (Figure 2.1). As the volcano is located in the north, soil of the northern farms originates from volcanic material. However, there is no volcanoes in the south, so all the soil from the southern farms were non-andic soils. Furthermore, as some volcanoes were recently active, soils with andic and partly andic properties were relatively younger than non-andic soils.

Table 2.2. Overview of the growing conditions of the different sampling farms in Costa Rica.

Farm	Altitude (m)	Distance from sea (km)	*Reventaz on* river position[a]	Rainfall[a]	Soil type composition (%) [a]				Texture stratum composition (%)[a]				Andic stratum composition (%)[a]		
					Fertile well-drained	Fertile poorly-drained	Infertile well-drained	Peat	Sand	Sandy loam	Clay loam	Clay	Andic	Part andic	Non-andic
1	41	38.27	1*	3500-4000	48	41	9	1	3	43	39	15	14	43	43
2	40	37.01	1*	3500-4000	48	52	0	0	12	24	40	24	8	40	52
3	62	47.11	1*	3500-4000	37	63	0	0	4	45	51	0	11	63	26
4	44	27.98	1*	3500-4000	74	25	0	0	6	61	33	0	29	57	14
5	62	34.45	1*	3500-4000	98	0	2	0	0	75	23	2	75	2	23
6	52	28.94	1*	3500-4000	58	42	0	0	9	73	18	0	0	92	8
7	44	30.03	1*	3500-4000	100	0	0	0	0	70	30	0	70	0	30
8	23	23.45	2#	3000-3500	100	0	0	0	0	100	0	0	0	100	0
9	33	23.35	2#	3000-3500	94	6	0	0	0	94	6	0	0	77	23
10	16	18.16	2#	3000-3500	12	88	0	0	0	36	64	0	0	84	16
11	19	18.90	2#	3500-4000	91	9	0	0	0	91	9	0	0	0	100
12	17	5.67	2#	3000-3500	64	36	0	0	0	12	88	0	0	0	100
13	8	10.66	2#	3000-3500	70	30	0	0	0	0	100	0	0	0	100
14	8	2.37	2#	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc
15	12	13.38	2	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc	nc

Farms located North (*) and South (#) of the *Reventazon* river; (%) Percentage; (nc) Not collected for analysis; [a] Adapted from Wesselink (2016).

2.3.2. Stable isotope compositions

2.3.2.1. Differences in bananas from different farms

Figure A.1. (Appendix A) shows the scatter plots of different stable isotope ratios, to illustrate the relationship between the growing conditions and the stable isotopes in the bananas. However, from the scatter plots no clear separation and/or groupings of samples were observed. The three kinds of stable isotopes were also determined in banana pulp and are presented as mean ± standard deviation in Table 2.3. The $\delta^{13}C$ values for the banana samples from all the farms range from -26.2 ± 0.3‰ for Farm 3 to -25.1 ± 0.6‰ for Farm 13, which is consistent with the results generally reported for C_3 plants where the ratio of isotopes is generally between -33 to -24 ‰ (O'Leary, 1988). The limited variation of the $\delta^{13}C$ values can further be explained by the fact that all the banana samples in the study were from the same variety (*Cavendish*) belonging to the C_3 crops group. More variation was observed for the nitrogen isotopes. The $\delta^{15}N$ values ranged from 0.8 ± 0.1‰ for Farm 14 to 3.5 ± 0.6‰ for Farm 5. The stable nitrogen isotope ratios are mainly dependant on the soil's nutrition as well as the fertiliser types used on the different farms. As reported, the $\delta^{15}N$ values will increase if the more organic fertilisers are used on the farm. It is evident that Farm 14 had significantly different $\delta^{15}N$ values compared to the other farms. This could be due to the use of less organic fertilisers, because a higher proportion of organic fertiliser tends to increase $\delta^{15}N$ values (Šturm, Kacjan-Maršić, & Lojen, 2011). For the $\delta^{18}O$ values, Farm 14 was observed to contain the highest values with 29.2 ± 0.5‰. Two other farms, Farm 7 (27.4 ± 0.3‰) and Farm 5 (27.3 ± 0.7‰), had significantly lower $\delta^{18}O$ values than Farm 14 ($P < 0.05$) (Table 2.3). This could be due to the distance from the sea as Farm 7 (30 km) and 5 (34 km) were located further than Farm 14 (2 km). This is in accordance with literature as it has been reported that the $\delta^{18}O$ values in precipitation is gradually reduced as it moves away from the coastline (Clark & Fritz, 1997). The $\delta^{18}O$ values of the other farms ranged from 27.7 ± 1.1‰ to 29.0 ± 0.7‰.

Table 2.3. The $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ mean ± standard deviation values of the banana pulp samples collected from the different farms in Costa Rica.

Farm	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)
1	6	$-25.6^{bcd} \pm 0.2$	$2.8^{bc} \pm 0.6$	$28.9^{abc} \pm 0.3$
2	5	$-25.5^{bcd} \pm 0.3$	$3.2^{ab} \pm 0.4$	$28.3^{bcde} \pm 0.6$
3	6	$-26.2^{e} \pm 0.3$	$2.5^{cd} \pm 0.5$	$28.5^{abcd} \pm 0.6$
4	6	$-25.4^{bcd} \pm 0.2$	$2.1^{d} \pm 0.7$	$28.2^{cde} \pm 0.4$
5	6	$-25.3^{bc} \pm 0.3$	$3.5^{a} \pm 0.6$	$27.3^{f} \pm 0.7$
6	6	$-25.8^{cde} \pm 0.3$	$2.1^{d} \pm 0.2$	$27.7^{ef} \pm 1.1$
7	5	$-25.9^{de} \pm 0.3$	$2.1^{d} \pm 0.6$	$27.4^{f} \pm 0.3$
8	6	$-25.2^{ab} \pm 0.4$	$2.6^{bcd} \pm 0.4$	$28.2^{cde} \pm 0.5$
9	6	$-25.6^{bcd} \pm 0.3$	$3.1^{abc} \pm 0.4$	$28.0^{def} \pm 0.4$
10	6	$-25.6^{bcd} \pm 0.2$	$3.5^{a} \pm 0.5$	$28.2^{cde} \pm 0.4$
11	6	$-25.5^{bcd} \pm 0.2$	$2.1^{d} \pm 0.4$	$28.6^{abcd} \pm 0.3$
12	6	$-25.5^{bcd} \pm 0.4$	$2.2^{d} \pm 0.7$	$28.0^{def} \pm 0.4$
13	6	$-25.1^{ab} \pm 0.6$	$2.7^{bcd} \pm 0.4$	$29.0^{ab} \pm 0.7$
14	6	$-24.8^{a} \pm 0.3$	$0.8^{e} \pm 0.1$	$29.2^{a} \pm 0.5$
15	6	$-26.1^{e} \pm 0.7$	$2.0^{d} \pm 0.3$	$28.8^{abc} \pm 0.2$

(n) Number of samples; $^{a-f}$ Different mean values in the same column with different superscript letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

2.3.2.2. The relationship between growing conditions and stable isotope compositions of bananas

Figure 2.2 shows the correlation between the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values and the growing conditions (i.e., rainfall, altitude, distance to sea, and other soil conditions on the other hand). The $\delta^{13}C$ values showed a stronger negative correlation with altitude ($r = -0.57$, $p = 0.04$) and distance from sea ($r = -0.55$, $p = 0.05$) than the other conditions. Compared with infertile and poorly drained soils, the FWD soil type could increase the $\delta^{13}C$ value. The reason can be ascribed to the fact that well drained soil conditions contribute to the activity of soil microbes, and then promotes the metabolism of plants, thereby enhancing photosynthesis and increasing the fixed activity of carbon dioxide. Normally, the difference in carbon compositions is mainly due to differences in varieties, as different species have different CO_2 fixation pathways. However, for the same variety, due to geographical differences and management systems, the latitude, altitude, light, fertiliser, precipitation, and other factors could also affect the fixed efficiency of carbon dioxide, and thus the proportion of carbon composition (Diefendorf, Mueller, Wing, Koch, & Freeman, 2010). Lagad et al. (2013) reported that the different $\delta^{13}C$ ratios in tea samples from four regions could be influenced by elevation, latitude,

and longitude (Lagad et al., 2013). However, compared with $\delta^{15}N$ and $\delta^{18}O$, $\delta^{13}C$ values show lower correlation with growing conditions as the carbon isotope abundance is more dependent on plant species than on the geographical origin (Ocvirk, Ogrinc, & Košir, 2018), which is in line with the current manuscript and reports in literature. For the variation of stable oxygen isotopes, the $\delta^{18}O$ values were moderately correlated with altitude (r = -0.46, p = 0.12) (Figure 2.2), which is consistent with the elevation and rainfall effects of the oxygen isotope distribution (Breitenbach et al., 2010). In a study determining the geographical origin of tomatoes from Slovenia, Italy, Spain, and Morocco, a similar trend in results were seen with $\delta^{18}O$ values negatively correlated with altitude (Opatić et al., 2018). The distance from the sea could also have weak negative effects on the $\delta^{18}O$ values (r = -0.07, p = 0.81), because in the process of water transfer from the ocean to the land, the heavy isotopes in the water always rain first in the place closer to the sea (Lachniet et al., 2007). Soil conditions such as clay proportion could increase the $\delta^{18}O$ values (r = 0.27, p = 0.37), which could be caused by high water holding activity in clay soil. The differences in rainfall also showed slight correlation with $\delta^{18}O$ values, the effects could be caused by the difference of 500 mm in two major precipitation distributions (Table 2.2).

Figure 2.2 shows that the $\delta^{15}N$ values were slightly correlated with rainfall (r = 0.24, p = 0.42), fertile well drained conditions (r = -0.27, p = 0.36), clay (r = 0.37, p = 0.37), and non-andic properties (r = -0.23, p = 0.44) in soils. The andic properties refer to the result of moderate weathering of mainly pyroclastic deposits (Parfitt & Clayden, 1991). This condition could be reflected in the soil origin because some soils develop andic properties from non-volcanic materials (e.g., loess, argillite, and ferralitic weathering products), and normally many surface layers with andic properties contain a high amount of organic matter (more than 5%) (Takahashi, Nanzyo, & Shoji, 2004). Highly organic matters could increase the ^{15}N percentage more than ^{14}N. However, it has also been reported that the difference of $\delta^{15}N$ values in plants are mainly caused by fertiliser conditions (Laursen et al., 2013). In the export farms of Costa Rica, a high rate of conventional fertilisers is applied every year due to the high productivity required. Besides, organic fertilisers such as chicken manure, compost, green manure, and bocashi could be included in the banana cultivation (Bellamy, 2013). Most of the $\delta^{15}N$ values did not show large differences because these farms followed similar fertilization strategies. However, Farm 14 and 15 presented lower $\delta^{15}N$ values. For the mineral

fertiliser production, the nitrogen from the atmosphere is chemically fixated by industrial catalytic reactions. Consequently, artificial fertiliser and plants fertilized with these products have $\delta^{15}N$ values close to 0‰, like in air. In contrast, the $\delta^{15}N$ value will be higher when organic fertilisers are applied because the animal origin fertilisers comprise 5‰ $\delta^{15}N$ since the animal metabolism increases the $\delta^{15}N$ values (Bateman, Kelly, & Jickells, 2005). According to the recent research progress of stable isotopes applications, the combined analysis of C, N, and O isotopes is a rapid and accurate method to link stable isotopic compositions to their growing conditions for the prevention of food fraud (Park, Choi, & Bong, 2019). It is advised that in future studies more stable isotopic types (e.g., $\delta^{2}H$, $\delta^{34}S$, $\delta^{87}Sr$) and their abundances from different plant bodies should be considered to reflect the effects of growing conditions more accurately (Camin et al., 2017).

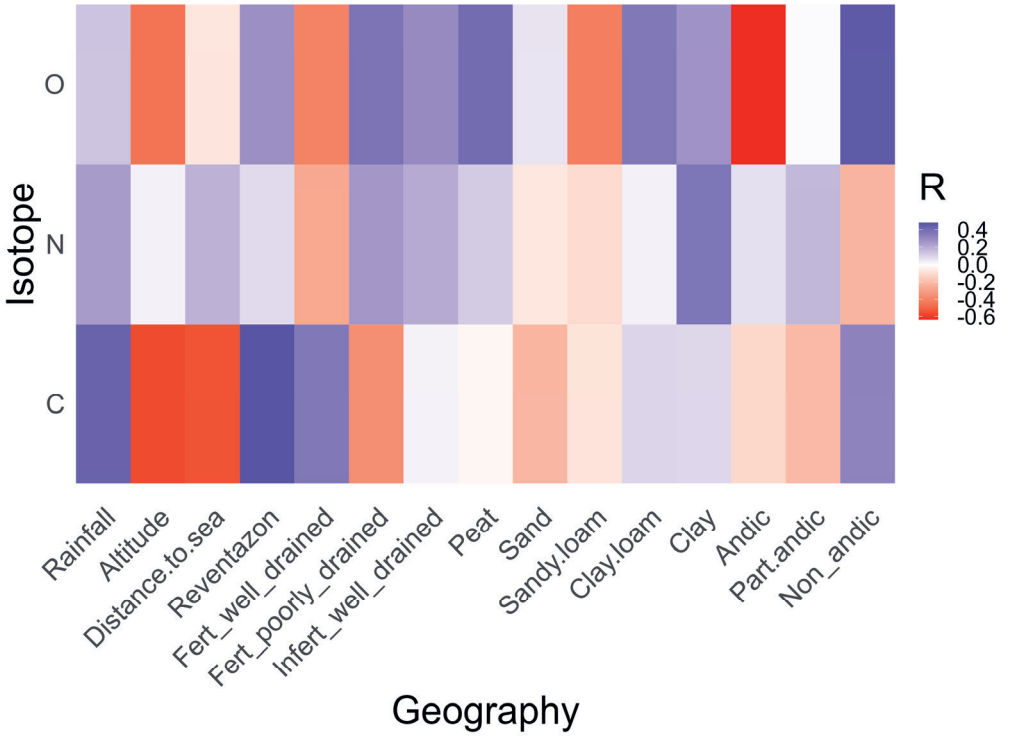


Figure 2.2. Correlation analyses between the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values and growing conditions. (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

2.3.3. Elemental compositions

2.3.3.1. Elemental concentrations in the banana samples

In total, 88 banana pulp and 88 banana peel samples were subjected to elemental analysis. The elemental results of banana pulp and peel are shown as means ± standard deviations in Table 2.4 and 2.5, respectively. A one-way ANOVA was used for determination of significant differences between the mean values of samples from different farms. Among these elements, macro-elements such as Ca, Mg, P, Fe, and micro-elements such as Co, Mn, Mo, Zn were detected. The similar elemental profiles were also reported in a previous study on pomegranate (Mphahlele et al., 2016), goji (Jeszka-Skowron, Zgoła-Grześkowiak, Stanisz, & Waśkiewicz, 2017), and mango (Sinha, Jaiswal, Ahmad, Mishra, & Sinha, 2017). In the pulp and peel samples, K, Ca, Mg, P, Mn, Fe were the main elements, which were followed by Al, Ba, Cu, Co, Sr etc.

In the peel samples, the highest mean concentration of Al, Ca, Mg, and Mo were detected for Farm 12 (Table 2.5). While, the lowest Cu and Sb content were found for Farm 12, and Farm 2 samples had the lowest concentration of Al, Mg, S, and Zn (Table 2.5). As reported in a comparison of fruits including pawpaw, pineapple, banana, and orange, Ca, Zn, Fe, and Mg were the main elements of banana peel (Dibanda et al., 2016). Similar elemental compositions were also determined in this research chapter's banana peel samples. Anhwange et al (2009) suggested that 10 kinds of elements such as P, Ca, Na, Fe, Mg, Br, Rb, Sr, Zr, and Nb formed the main mineral element map for the peel of banana (*Musa sapientum*) from Nigeria. In the current study, however, more elements such as K, Mn, Li, Ni, Si etc. appeared relevant from the current data. These differences among elemental compositions of bananas from different sources could be due to variation of the growing conditions such as rainfall, soil, and temperature.

Table 2.4. The mean ± standard deviation of the elemental compositions (mg/kg) of the banana pulp samples collected from the different farms in Costa Rica.

Farm	n	Al	Ba	Ca	Co	Cr	Cu	Fe	K	Li	Mg	Mn	Mo
1	6	0.7^{d}±1.0	3.3^{a}±0.9	331.8^{bc}±44.3	0.8^{d}±0.1	0.4^{c}±0.0	4.0^{def}±0.3	14.0^{b}±1.3	13993.6^{cd}±458.5	0.1^{d}±0.0	1188.5^{de}±73.4	6.1^{gh}±2.8	nd
2	6	0.7^{bcd}±0.8	3.0^{a}±0.7	307.4^{bcde}±34.4	0.8^{d}±0.1	0.4^{bc}±0.0	4.3^{bcdef}±0.3	14.8^{b}±0.6	16129.2^{a}±675.2	0.1^{d}±0.0	1304.4^{ab}±66.9	10.9^{def}±4.4	nd
3	6	1.3^{bcd}±2.0	1.4^{de}±0.2	314.6^{bcd}±61.9	0.9^{d}±0.0	0.5^{bc}±0.1	5.2^{ab}±0.5	17.5^{b}±1.2	16007.2^{a}±871.6	0.1^{d}±0.1	1372.4^{a}±82.9	7.2^{fgh}±1.7	nd
4	8	1.7^{bcd}±1.0	2.6^{abc}±0.3	243.5^{f}±42.8	1.6^{ab}±0.1	0.6^{abc}±0.1	5.0^{abc}±1.1	15.3^{b}±6.4	14335.5^{cd}±729.8	0.7^{ab}±0.0	1236.6^{bcd}±72.1	10.2^{efg}±1.9	0.3^{ab}±0.1
5	6	2.2^{abcd}±0.9	3.3^{a}±0.4	259.8^{ef}±25.7	1.1^{c}±0.4	0.4^{bc}±0.0	4.3^{bcdef}±0.6	15.6^{b}±1.3	15635.3^{ab}±568.9	0.3^{c}±0.2	1180.5^{de}±94.1	10.5^{bcde}±2.6	0.11^{cde}±0.1
6	6	2.8^{abc}±4.0	2.9^{ab}±1.4	347.8^{b}±35.1	0.8^{d}±0.0	0.9^{ab}±1.3	5.2^{ab}±1.0	18.1^{b}±5.3	13701.6^{d}±484.3	0.1^{d}±0.0	1282.7^{bc}±55.0	12.5^{cde}±4.5	nd
7	6	0.2^{cd}±1.1	2.7^{ab}±0.8	284.1^{cdef}±49.1	0.8^{d}±0.1	0.4^{bc}±0.0	5.2^{a}±0.2	14.5^{b}±1.1	14813.5^{bc}±941.2	0.1^{d}±0.0	1276.8^{bc}±60.1	16.9^{ab}±3.8	nd
8	6	2.2^{abcd}±1.4	1.4^{de}±0.2	270.0^{def}±58.4	1.5^{ab}±0.1	0.8^{abc}±0.4	3.8^{f}±0.4	17.6^{b}±2.9	14514.1^{cd}±599.0	0.6^{b}±0.1	1136.4^{e}±26.2	9.5^{efgh}±2.7	0.4^{ab}±0.0
9	6	0.27^{d}±0.5	0.9^{e}±0.1	280.4^{cdef}±34.3	0.8^{d}±0.1	0.4^{bc}±0.0	3.7^{f}±0.5	14.7^{b}±1.0	14860.6^{bc}±744.3	0.1^{d}±0.0	1238.7^{bcd}±70.4	5.8^{h}±1.0	nd
10	6	4.5^{a}±3.6	1.7^{d}±0.2	278.4^{def}±19.7	1.7^{a}±0.1	0.9^{abc}±0.2	4.7^{abcde}±0.5	30.5^{a}±28.9	13829.9^{cd}±672.5	0.7^{ab}±0.1	1165.5^{de}±37.5	15.2^{abcd}±5.6	0.5^{a}±0.1
11	6	2.7^{abc}±0.4	1.3^{de}±0.0	353.9^{b}±14.3	1.6^{ab}±0.0	1.0^{a}±0.4	3.9^{ef}±0.2	18.5^{b}±3.6	13764.7^{d}±538.5	0.6^{b}±0.0	1205.5^{cde}±57.8	15.7^{abc}±4.2	0.3^{ab}±0.2
12	6	0.8^{bcd}±1.4	1.9^{cd}±0.4	415.4^{a}±46.6	0.8^{d}±0.1	0.4^{c}±0.0	4.1^{cdef}±0.4	13.2^{b}±0.9	14124.8^{cd}±1379.1	0.1^{d}±0.0	1280.0^{bc}±72.7	17.3^{ab}±1.6	0.1^{bcd}±0.6
13	6	3.0^{ab}±2.3	2.1^{bcd}±0.3	281.5^{cdef}±34.1	1.7^{a}±0.1	0.7^{abc}±0.1	$5.2a^{b}$±0.8	15.4^{b}±3.9	13596.3^{d}±773.0	0.7^{b}±0.0	1217.7^{bcde}±48.2	19.4^{a}±5.1	0.3^{abc}±0.1
14	6	1.8^{bcd}±2.1	2.7^{ab}±0.5	261.9^{def}±31.6	1.6^{ab}±0.1	0.6^{abc}±0.0	4.9^{abcd}±1.2	15.3^{b}±1.8	14565.1^{cd}±850.0	0.7^{ab}±0.1	1137.2^{e}±53.4	10.6^{efg}±2.1	0.5^{a}±0.2
15	6	3.0^{ab}±1.0	1.8^{cd}±0.2	249.8^{f}±22.1	1.4^{b}±0.0	0.4^{bc}±0.0	5.2^{a}±0.5	14.2^{b}±1.2	14519.0^{cd}±428.6	0.8^{a}±0.0	1242.6^{bcd}±66.0	13.9^{bcde}±3.0	0.5^{a}±0.1

[a-i] Different mean values in the same column with different superscript letters are significantly different ($P < 0.05$) according to Duncan's multiple range test; (n) Number of samples; (nd) Not detectable.

Table 2.4. (continued)

Farm	n	Na	Ni	P	Pb	S	Sb	Sc	Si	Sr	Y	Zn
1	6	242.5^{bc}±103.5	1.3^{cde}±0.1	971.9^{cd}±77.0	0.4^{d}±0.4	356.1^{e}±16.7	nd	0.1^{f}±0.0	215.0^{ab}±53.7	3.7^{cde}±0.6	0.1^{d}±0.0	8.9^{cd}±1.9
2	6	256.5^{ab}±78.5	1.4^{bcde}±0.1	945.3^{cde}±44.8	0.5^{d}±0.4	356.9^{e}±12.3	nd	0.1^{ef}±0.0	254.7^{a}±32.0	3.8^{bcde}±0.3	0.1^{d}±0.0	9.1^{cd}±2.2
3	6	319.8^{a}±93.6	1.4^{bcde}±0.2	959.6^{cde}±59.6	0.5^{d}±0.3	439.4^{a}±56.7	nd	0.2^{cdef}±0.0	224.1^{ab}±50.7	2.7^{f}±0.3	0.2^{d}±0.0	9.4^{bcd}±2.5
4	8	133.6^{d}±28.3	1.4^{bcde}±0.1	965.0^{cd}±56.6	1.7^{abc}±0.7	397.6^{bcd}±18.3	0.8^{a}±0.5	0.2^{cd}±0.0	228.3^{ab}±32.4	4.4^{bc}±0.6	0.6^{b}±0.0	12.0^{bc}±3.7
5	6	138.3^{d}±12.8	1.4^{bcde}±0.2	901.5^{def}±83.2	1.0^{d}±0.6	399.0^{bcd}±33.0	nd	0.2^{cdef}±0.0	185.4^{b}±50.4	3.7^{cde}±0.7	0.3^{c}±0.2	10.6^{bcd}±4.2
6	6	206.3^{bcd}±43.5	1.4^{bcde}±0.1	1005.7^{bc}±28.4	0.5^{d}±0.3	397.3^{bcd}±33.0	nd	0.1^{ef}±0.0	260.8^{a}±19.3	4.0^{bcd}±0.9	0.1^{d}±0.0	10.6^{bcd}±3.1
7	6	151.1^{d}±8.7	1.2^{e}±0.1	1006.3^{bc}±62.5	0.3^{d}±0.4	406.2^{abcd}±21.6	0.6^{ab}±0.3	0.1^{ef}±0.0	250.3^{a}±36.3	6.0^{a}±1.1	0.1^{d}±0.0	8.4^{cd}±1.0
8	6	142.3^{d}±22.5	1.3^{de}±0.1	910.2^{cdef}±31.4	1.4^{bc}±0.6	416.5^{ab}±20.2	nd	0.2^{cde}±0.0	226.4^{ab}±35.6	3.6^{cde}±0.7	0.6^{b}±0.0	9.7^{bcd}±1.4
9	6	153.0^{d}±10.6	1.5^{abcd}±0.0	869.8^{ef}±62.3	0.6^{d}±0.4	371.0^{de}±23.7	nd	0.1^{def}±0.0	227.3^{ab}±17.5	2.6^{f}±0.1	0.1^{d}±0.0	8.0^{d}±0.6
10	6	154.9^{d}±56.5	1.8^{a}±0.4	985.7^{bcd}±36.9	1.7^{ab}±0.5	391.3^{bcd}±31.9	nd	0.2^{cd}±0.0	259.7^{a}±38.5	4.0^{bcd}±0.3	0.6^{b}±0.0	12.9^{b}±3.9
11	6	272.0^{ab}±136.1	1.6^{ab}±0.2	986.7^{bcd}±11.3	1.7^{abc}±0.6	412.4^{abc}±8.1	nd	0.2^{c}±0.0	226.5^{ab}±11.6	3.0^{ef}±0.2	0.6^{b}±0.0	26.5^{a}±3.4
12	6	155.2^{d}±17.0	1.3^{cde}±0.2	1153.2^{a}±168.4	0.4^{d}±0.4	378.2^{cde}±31.9	nd	0.1^{def}±0.0	258.2^{a}±44.2	3.2^{ef}±0.3	0.1^{d}±0.0	8.2^{d}±0.7
13	6	177.7^{cd}±33.1	1.5^{bcde}±0.1	925.0^{cde}±36.9	2.1^{a}±0.0	413.1^{abc}±7.5	nd	0.2^{bc}±0.0	243.4^{a}±26.9	3.3^{def}±0.1	0.6^{b}±0.0	10.9^{bcd}±1.7
14	6	140.3^{d}±22.1	1.6^{abc}±0.2	830.5^{f}±55.7	1.7^{abc}±0.9	421.3^{ab}±23.3	nd	0.2^{b}±0.1	240.5^{a}±42.2	5.5^{a}±0.7	0.6^{b}±0.0	10.6^{bcd}±1.2
15	6	148.0^{d}±20.6	1.6^{ab}±0.1	1072.1^{ab}±88.8	0.7^{d}±0.2	354.3^{e}±13.3	0.9^{a}±0.6	0.5^{a}±0.0	222.1^{ab}±32.3	4.5^{b}±0.4	0.8^{a}±0.0	12.0^{bc}±4.4

$^{a\text{-}i}$ Different mean values in the same column with different superscript letters are significantly different ($P < 0.05$) according to Duncan's multiple range test; (n) Number of samples; (nd) Not detectable.

Table 2.5. The mean ± standard deviation elemental compositions (mg/kg) of the banana peel samples collected from the different farms in Costa Rica.

Farm	n	Al	Ba	Ca	Co	Cr	Cu	Fe	K	Li	Mg	Mn
1	6	15.9^{bcd}±3.5	16.8^{a}±4.8	2944.4^{abc}±673.3	0.9^{ef}±0.0	0.7^{a}±0.3	3.8^{efg}±0.6	30.4^{b}±8.1	51039.5^{ef}±2752.2	0.1^{d}±0.0	1228.9^{bc}±183.5	21.5^{hi}±7.8
2	6	4.1^{e}±2.4	14.8^{ab}±4.5	2495.9^{bcde}±547.9	0.9^{ef}±0.1	0.4^{bc}±0.1	2.9^{h}±0.5	25.6^{b}±1.7	52926.5^{ef}±6363.6	0.1^{d}±0.1	818.2^{f}±105.6	34.61^{defgh}±14.3
3	6	5.8^{e}±2.3	6.4^{dce}±1.4	2629.0^{abcd}±427.7	0.9^{ef}±0.1	0.4^{bc}±0.1	3.4^{fgh}±0.5	26.6^{b}±2.0	56611.4^{cde}±5470.2	0.1^{d}±0.0	889.9^{f}±155.8	25.1^{ghi}±6.7
4	8	15.0^{bcde}±4.0	10.7^{bc}±1.9	1835.0^{e}±379.5	1.3^{bc}±0.1	0.5^{bc}±0.0	5.3^{ab}±0.7	21.5^{b}±1.1	53983.5^{def}±4254.4	0.8^{a}±0.0	929.0^{ef}±173.9	32.2^{efghi}±6.4
5	6	7.8^{de} ±3.1	16.2^{a}±4.5	2621.3^{abcd}±349.3	1.1^{cd}±0.2	0.5^{bc}±0.1	4.1^{de}±0.7	26.6^{b}±5.8	66506.5^{a}±8163.3	0.4^{c}±0.2	955.3^{ef}±108.4	35.6^{defg}±9.7
6	6	14.0^{bcde}±4.4	14.4^{ab}±8.9	2807.3^{abcd}±856.0	1.0^{de}±0.1	0.4^{bc}±0.0	3.8^{efg}±0.3	27.5^{b}±2.2	49485.8^{f}±2083.6	0.1^{d}±0.0	1424.6^{ab}±261.5	40.0^{cdef}±12.5
7	6	22.6^{bc}±3.3	13.2^{ab}±5.2	2664.9^{abcd}±666.5	0.9^{ef}±0.1	0.3^{c}±0.1	4.0^{efg}±0.4	26.7^{b}±1.9	56520.7^{cde}±3254.8	0.2^{d}±0.0	1244.9^{bc}±170.8	57.3^{a}±7.4
8	6	14.2^{bcde}±10.7	4.0^{e}±1.0	2665.5^{abcd}±706.5	1.4^{a}±0.1	0.4^{bc}±0.0	3.2^{gh}±0.3	19.4^{b}±1.4	63534.7^{ab}±1616.1	0.6^{b}±0.0	1014.4^{def}±129.7	29.7^{fghi}±6.9
9	6	14.4^{bcde}±21.8	4.1^{e}±0.4	2993.0^{ab}±548.7	0.8^{f}±0.1	0.6^{ab}±0.3	3.2^{gh}±0.2	53.0^{a}±69.2	62275.7^{abc}±2801.6	0.3^{cd}±0.1	1104.7^{cde}±81.2	20.7^{i}±3.2
10	6	8.9^{de}±2.3	5.6^{de}±1.3	2483.0^{bcde}±501.3	1.3^{ab}±0.1	0.4^{bc}±0.1	4.9^{abc}±0.5	19.1^{b}±1.2	56439.2^{cde}±2493.2	0.6^{ab}±0.2	1117.4^{cde}±156.4	46.3^{abcd}±16.3
11	6	14.2^{bcde}±4.1	3.4^{e}±0.7	2599.5^{abcd}±596.3	1.2^{bc}±0.1	0.5^{bc}±0.0	4.6^{bcd}±0.3	22.1^{b}±5.1	55910.8^{de}±5169.4	0.7^{ab}±0.0	1243.3^{bc}±155.4	44.0^{bcde}±11.9
12	6	32.9^{a}±16.5	7.9^{cde}±2.4	3259.0^{a}±520.7	0.9^{ef}±0.1	0.4^{bc}±0.1	2.9^{h}±0.3	24.7^{b}±2.6	51368.0^{ef}±6580.0	0.2^{d}±0.0	1530.8^{a}±207.5	53.0^{ab}±8.2
13	6	8.0^{de}±3.0	7.4^{cde}±1.3	2226.9^{cde}±353.0	1.3^{bc}±0.1	0.4^{bc}±0.0	4.8^{abc}±0.5	21.0^{b}±1.2	55106.4^{def}±2697.3	0.7^{ab}±0.1	1196.1^{cd}±207.9	55.8^{ab}±13.5
14	6	11.8b^{cde}±4.3	10.2^{bcd}±3.1	2102.7^{de}±303.0	1.3^{ab}±0.0	0.5^{bc}±0.0	5.4^{a}±0.3	24.5^{b}±1.7	59198.9^{bcd}±2675.1	0.8^{ab}±0.0	967.4^{ef}±144.8	33.1^{efghi}±8.1
15	6	24.9^{ab}±3.9	4.7^{e}±0.4	2888.7^{abc}±331.0	1.3^{bc}±0.1	0.4^{bc}±0.0	4.6^{cd}±0.7	30.1^{b}±12.5	63445.3^{ab}±7083.2	0.6^{b}±0.2	1275.3^{bc}±120.1	48.6^{abc}±8.6

$^{a-f}$ Different mean values in the same column with different superscript letters are significantly different ($P < 0.05$) according to Duncan's multiple range test; (n) Number of samples; (nd) Not detectable.

Table 2.5 (continued)

Farm	n	Mo	Na	Ni	P	Pb	S	Sb	Sc	Si	Sr	Y	Zn
1	6	$1.3^{b}\pm0.1$	$86.1^{d}\pm8.2$	$1.1^{efg}\pm0.1$	$2067.6^{abcd}\pm418.2$	nd	$561.9^{cdef}\pm26.1$	nd	nd	$704.6^{bc}\pm236.7$	$30.3^{bcd}\pm5.2$	$0.2^{d}\pm0.1$	$15.7^{bc}\pm3.4$
2	6	$1.1^{b}\pm0.1$	$99.5^{cd}\pm13.3$	$1.0^{fg}\pm0.0$	$1698.1^{e}\pm330.8$	$0.4^{cdef}\pm0.3$	$507.3^{f}\pm68.6$	$0.4^{bcd}\pm0.4$	nd	$1028.4^{a}\pm362.5$	$29.4^{cd}\pm4.7$	$0.3^{d}\pm0.0$	$14.3^{c}\pm2.4$
3	6	$1.2^{b}\pm0.0$	$94.2^{d}\pm6.6$	$0.9^{g}\pm0.2$	$2366.4^{a}\pm238.4$	$0.4^{def}\pm0.7$	$643.4^{ab}\pm43.3$	$0.6^{abc}\pm0.8$	nd	$611.5^{bcd}\pm218.8$	$19.2^{f}\pm3.8$	$0.2^{d}\pm0.0$	$15.8^{bc}\pm2.3$
4	8	$0.7^{bc}\pm0.1$	$158.6^{bcd}\pm14.9$	$1.6^{ab}\pm0.1$	$2053.1^{abcd}\pm171.0$	$1.2^{a}\pm0.4$	$561.4^{cdef}\pm40.9$	$1.2^{ab}\pm0.6$	$0.5^{a}\pm0.0$	$367.3^{de}\pm40.6$	$31.3^{bcd}\pm4.9$	$0.7^{a}\pm0.0$	$17.2^{bc}\pm2.0$
5	6	nd	$321.4^{a}\pm147.6$	$1.3^{cd}\pm0.2$	$2247.8^{ab}\pm244.6$	$1.2^{a}\pm0.4$	$608.9^{abc}\pm71.1$	nd	$0.2^{c}\pm0.0$	$569.2^{bcd}\pm67.1$	$34.4^{bc}\pm7.1$	$0.3^{d}\pm0.2$	$18.7^{bc}\pm4.8$
6	6	$1.1^{b}\pm0.0$	$94.0^{d}\pm6.9$	$1.3^{cde}\pm0.2$	$1775.5^{de}\pm197.6$	$0.0^{fg}\pm0.3$	$590.4^{bcd}\pm56.5$	$0.6^{abcd}\pm0.7$	nd	$762.8^{b}\pm105.7$	$31.7^{bcd}\pm11.6$	$0.2^{d}\pm0.0$	$17.2^{bc}\pm3.7$
7	6	$1.0^{b}\pm0.0$	$95.5^{d}\pm3.6$	$1.0^{efg}\pm0.1$	$2137.3^{abc}\pm162.3$	$0.6^{bcde}\pm0.2$	$661.9^{a}\pm29.4$	$0.6^{abc}\pm0.5$	nd	$801.7^{ab}\pm284.0$	$55.9^{a}\pm12.5$	$0.2^{d}\pm0.0$	$17.4^{bc}\pm3.2$
8	6	$0.4^{bc}\pm0.2$	$151.4^{bcd}\pm38.9$	$1.3^{cd}\pm0.1$	$1746.5^{de}\pm300.9$	$1.3^{a}\pm0.4$	$580.5^{bcde}\pm42.6$	$0.5^{abc}\pm0.4$	$0.2^{c}\pm0.0$	$778.4^{b}\pm90.7$	$25.8^{def}\pm6.4$	$0.5^{c}\pm0.0$	$17.3^{bc}\pm2.9$
9	6	nd	$292.0^{a}\pm144.8$	$1.4^{bc}\pm0.3$	$2131.9^{abc}\pm525.7$	$0.7^{abcd}\pm0.6$	$524.9^{def}\pm49.4$	$0.5^{abcd}\pm0.5$	$0.1^{c}\pm0.2$	$588.3^{bcd}\pm246.8$	$23.2^{def}\pm1.5$	$0.2^{d}\pm0.0$	$15.5^{bc}\pm0.8$
10	6	$0.6^{bc}\pm0.4$	$147.3^{bcd}\pm29.0$	$1.6^{ab}\pm0.0$	$1962.9^{bcde}\pm143.2$	$0.8^{abcd}\pm0.5$	$515.8^{ef}\pm51.6$	$0.7^{abc}\pm0.8$	$0.3^{b}\pm0.2$	$498.3^{cde}\pm176.5$	$30.7^{bcd}\pm5.0$	$0.6^{bc}\pm0.1$	$15.6^{bc}\pm2.4$
11	6	$0.4^{bc}\pm0.1$	$155.7^{bcd}\pm32.0$	$1.7^{a}\pm0.1$	$1759.7^{de}\pm135.6$	$1.1^{ab}\pm0.1$	$531.9^{def}\pm25.8$	$0.6^{abc}\pm0.6$	$0.5^{a}\pm0.0$	$440.5^{de}\pm61.4$	$18.4^{f}\pm4.0$	$0.7^{ab}\pm0.0$	$16.8^{bc}\pm1.9$
12	6	$2.4^{a}\pm2.6$	$96.6^{d}\pm6.7$	$1.2^{def}\pm0.1$	$1864.7^{cde}\pm237.5$	$0.1^{efg}\pm0.2$	$645.4^{ab}\pm50.1$	nd	nd	$816.6^{ab}\pm164.2$	$23.2^{def}\pm5.4$	$0.2^{d}\pm0.0$	$17.4^{bc}\pm2.6$
13	6	$0.3^{bc}\pm0.1$	$178.1^{b}\pm49.7$	$1.6^{ab}\pm0.1$	$1620.0^{e}\pm194.6$	$0.9^{abcd}\pm0.5$	$565.6^{cdef}\pm71.7$	$1.5^{a}\pm0.4$	$0.5^{a}\pm0.0$	$380.6^{de}\pm113.6$	$20.5^{ef}\pm2.6$	$0.7^{ab}\pm0.0$	$17.3^{bc}\pm1.5$
14	6	$0.4^{bc}\pm0.0$	$173.4^{bc}\pm10.5$	$1.6^{ab}\pm0.1$	$1688.0^{e}\pm132.2$	$1.0^{abc}\pm0.2$	$621.9^{abc}\pm65.1$	$1.2^{ab}\pm0.5$	$0.5^{a}\pm0.0$	$319.0^{e}\pm86.1$	$38.4^{b}\pm7.6$	$0.7^{ab}\pm0.0$	$19.1^{bc}\pm3.9$
15	6	$0.8^{bc}\pm0.5$	$183.0^{b}\pm21.0$	$1.5^{bc}\pm0.2$	$2236.6^{ab}\pm145.5$	$0.9^{abcd}\pm0.2$	$593.1^{bcd}\pm65.1$	$0.8^{abc}\pm0.9$	$0.3^{b}\pm0.2$	$464.7^{cde}\pm203.6$	$27.8^{cde}\pm3.0$	$0.6^{c}\pm0.1$	$24.4^{a}\pm3.5$

[a-f] Different mean values in the same column with different superscript letters are significantly different ($P < 0.05$) according to Duncan's multiple range test; (n) Number of samples; (nd) Not detectable.

Among all the sampled farms, K, Ca, Mg, P, Mn, Fe were the main elemental compounds in banana pulp samples. Our findings regarding the elemental profiles of pulp samples are consistent with previous work. As reported by Wall et al. (2006), who studied the mineral composition of banana (*Musa* spp.) cultivars grown in Hawaii, P, Ca, Mg, Mn, and Zn were considered to be the main mineral elements in the banana pulp. In our research, high concentrations of Zn were determined in the pulp samples from Farm 4, 5, 6, 10, 11, and 15. Meanwhile, K was also found in high concentrations in pulp samples. Its concentration ranged from 13701.6 ± 484.3 mg/kg to 16129.2 ± 675.2 mg/kg which is also consistent with results for Malaysian bananas (Sulaiman et al., 2011).

2.3.3.2. Elemental concentrations in soil samples

Exchangeable Al, Ca, Mg, K, P, Zn, Cu, Fe, and Mn of soil samples were provided by the research division of the National Banana Corporation (Corbana) in Costa Rica. The levels of elements were relatively higher than those of other elements and they are essential for the growth of banana plants. The P, Ca, Cu, Zn, and Mg concentrations in soil samples varied considerably. For instance, the P content was reported as 18.9 ± 8.1 mg/mL for Farm 6, but the highest was reported as 93.0 ± 25.7 mg/mL for Farm 13. The concentration of Ca varied from 4.3 ± 0.6 for Farm 15 to 26.4 ± 2.3 for Farm 10. Other elements, such as K, Mg, and Fe, showed relatively smaller fluctuations. A detailed overview of the elemental compositions of the soil is provided in Table 2.6. The distribution of elements across the farms differed, which could be due to local growing conditions especially soil properties and rainfall (Stein et al., 2017; Wang, Takematsu, & Ambe, 2000), as well as fertilization practices.

Table 2.6. The mean values for the elemental composition of the soil samples collected from the different farms in Cost Rica.

Farm	n	Ca (cmol(+)/L)	Cu (mg/L)	Fe (mg/L)	K (cmol(+)/L)	Mg (cmol(+)/L)	Mn (mg/L)	P (mg/L)	Zn (mg/L)
3	12	$16.6^{e}\pm1.6$	$16.1^{a}\pm1.5$	$195.0^{d}\pm75.7$	$0.9^{c}\pm0.2$	$3.8^{de}\pm0.5$	$12.3^{fg}\pm5.9$	$54.8^{cd}\pm5.5$	$18.5^{a}\pm12.5$
4	11	$4.3^{i}\pm0.6$	$2.9^{e}\pm1.0$	$47.0^{f}\pm14.0$	$0.6^{c}\pm0.1$	$1.6^{g}\pm0.4$	$6.0^{g}\pm0.0$	$33.4^{d}\pm12.2$	$0.8^{e}\pm0.1$
5	36	$7.5^{gh}\pm3.2$	$3.8^{e}\pm1.9$	$149.9^{e}\pm62.0$	$1.5^{b}\pm0.4$	$2.6^{f}\pm0.8$	$17.2^{ef}\pm6.9$	$53.6^{cd}\pm19.5$	$8.7^{bc}\pm2.8$
6	36	$8.9^{h}\pm2.4$	$3.3^{e}\pm1.0$	$146.7^{e}\pm49.5$	$1.0^{c}\pm0.4$	$3.4^{e}\pm0.9$	$26.5^{de}\pm14.6$	$18.9^{e}\pm8.1$	$5.6^{bc}\pm2.6$
7	49	$9.6^{g}\pm2.4$	$3.7^{e}\pm1.5$	$147.5^{e}\pm58.6$	$0.9^{c}\pm0.5$	$2.6^{f}\pm0.8$	$33.4^{d}\pm19.3$	$39.4^{d}\pm33.2$	$10.0^{b}\pm7.3$
9	68	$13.5^{f}\pm2.5$	$5.6^{d}\pm0.9$	$248.8^{c}\pm21.9$	$1.8^{b}\pm0.9$	$4.1^{d}\pm0.4$	$24.3^{de}\pm8.1$	$87.5^{ab}\pm32.3$	$9.3^{b}\pm4.2$
10	47	$26.4^{a}\pm2.3$	$8.5^{b}\pm1.6$	$262.0^{bc}\pm26.2$	$1.6^{b}\pm0.5$	$8.3^{a}\pm1.0$	$56.8^{c}\pm16.3$	$86.8^{ab}\pm30.9$	$9.0^{b}\pm3.3$
11	31	$23.8^{b}\pm2.4$	$7.0^{c}\pm1.3$	$292.9^{b}\pm32.2$	$1.6^{b}\pm0.4$	$7.3^{b}\pm1.4$	$80.0^{a}\pm19.6$	$63.8^{c}\pm19.5$	$9.4^{b}\pm3.2$
12	25	$21.3^{c}\pm1.6$	$6.0^{d}\pm0.6$	$275.5^{bc}\pm23.4$	$0.6^{c}\pm0.2$	$6.5^{c}\pm0.8$	$49.8^{c}\pm13.5$	$41.0^{d}\pm11.4$	$4.9^{d}\pm1.2$
13	49	$19.3^{d}\pm2.2$	$5.8^{d}\pm0.8$	$332.6^{a}\pm38.2$	$1.6^{b}\pm0.5$	$7.0^{bc}\pm0.9$	$68.0^{b}\pm15.8$	$93.0^{a}\pm25.7$	$9.2^{b}\pm3.4$
14	15	$22.1^{c}\pm1.7$	$7.8^{bc}\pm1.0$	$328.9^{a}\pm36.6$	$2.4^{a}\pm1.5$	$6.9^{bc}\pm0.6$	$73.6^{ab}\pm27.5$	$71.6^{bc}\pm22.1$	$16.8^{a}\pm4.4$

(n) Number of samples; $^{a\text{-}i}$ Different mean values in the same column with different superscript letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

2.3.3.3. The relationships between elemental concentrations in banana, soil, and growing conditions

Principal component analysis (PCA) was performed to visualize sample groupings of banana samples according to soil andic properties of the origin. Figure 2.3 shows the PCA plots of bananas by geographical region based on the elemental composition in pulp (A), peel (B), and soil (C). For the pulp samples, mainly two sub-groups could be visualized using PCA. The same differences were reflected for the peel and soil results, which indicates the effect of geographical variation due to the presence of volcanoes. The biggest difference between the farms could be attributed to the presence of volcanoes in the north. The PCA results further proved the role of volcanoes on the composition of banana mineral elements. For the soil PCA results (Figure 2.3C), Farm 6, 11, 12, 13, 14 were clearly separated from other farms. These five farms were located in the south Atlantic zone. The same groupings were found for the PCA of pulp and peel samples. Because more elements were taken into accounts, it is possible to observe a good differentiation by contribution of Si, Mo, Ba, P, Ca, K, Cu, Sc, Pb, Sb,

and Li. Overall, PCA results further explained the important effects of growing conditions on the elemental composition of banana. In recently published papers, the minerals contents such as Pb, Cu, Ba, Mg, Rb, etc. were reported to be highly related to the geographical origin of tea (Zhao, Yu, & Li, 2017), grape seeds (Canizo, Escudero, Pérez, Pellerano, & Wuilloud, 2018), honey (Berriel, Barreto, & Perdomo, 2019), and other food materials. Although the selected elements varied between the different samples, the literature generally agrees on the critical role of elements in geographical origin discrimination.

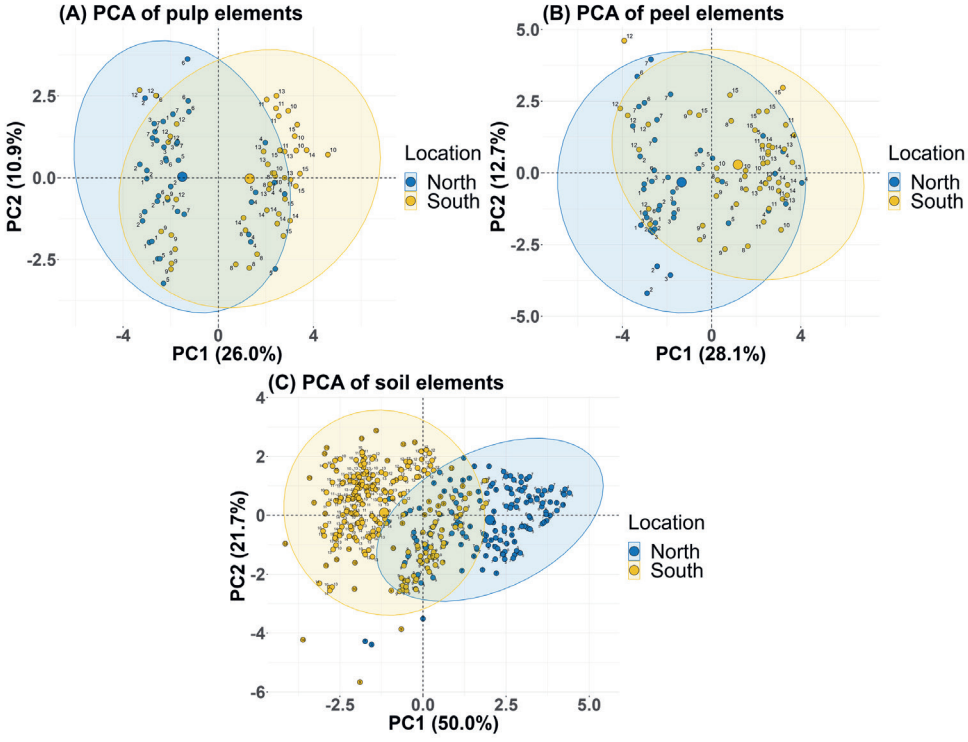


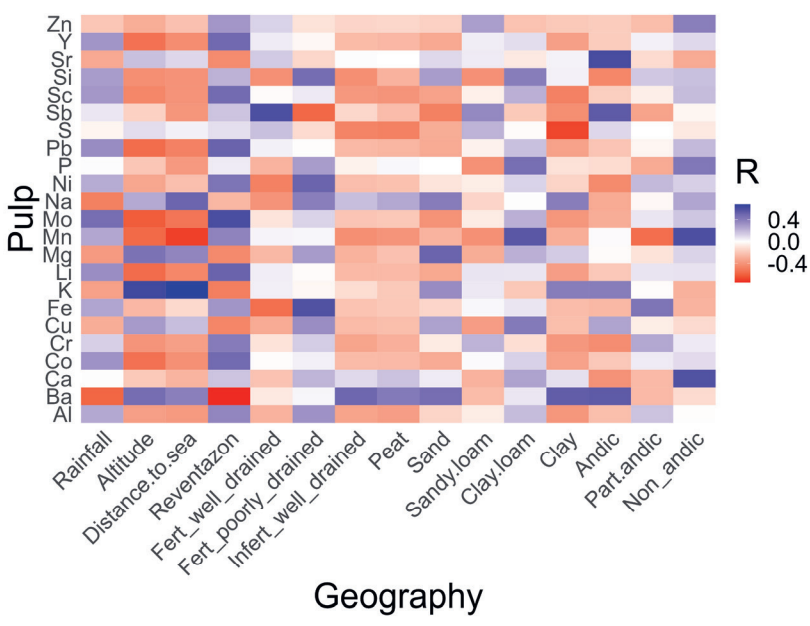
Figure 2.3. Principal component analysis (PCA) plot of the banana pulp (A), banana peel (B), and soil samples (C) collected from the different farms in Costa Rica. (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

Figure 2.4 presents the correlation between elemental compositions of banana, soil samples, and growing conditions. Pearson correlation analysis was employed for evaluating linear relationships of elements between the elemental composition of pulp,

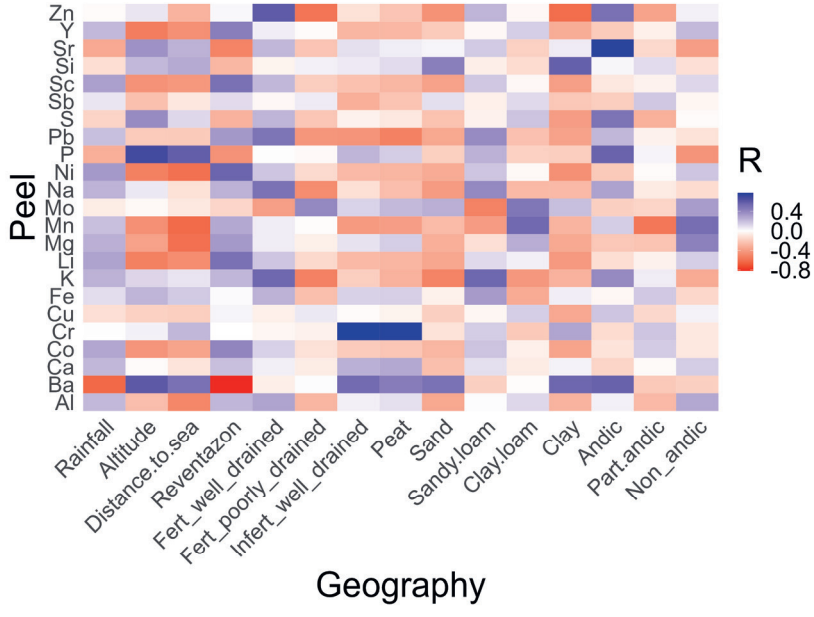
peel, soil samples on the one hand and growing conditions on the other hand. In general, the effect of growing conditions on different elements were distinctive. For the pulp samples, rainfall has slight effects for most of the elements, but is likely the main contributing factor (r = -0.59, p = 0.03) to decrease the level of Ba. Compared with rainfall, altitude could promote the level of K (r = 0.63, p = 0.02) and decrease the level of Li (r = -0.57, p = 0.04), Mn (r = -0.58, p = 0.04), and Mo (r = -0.62, p = 0.02) significantly. The main effects of the FWD soil type were opposite to FPD soil. For instance, for Fe, the former significantly inhibited the absorption of Fe (r = -0.55, p = 0.05), while the latter promoted its level (r = 0.58, p = 0.04). This could be caused by effects of water in soil on elemental absorption. Well-drained soil type could avoid the concentration of mineral elements in the soil, to avoid the absorption effect caused by root dehydration (Alloway, 2013). Therefore, banana pulp samples from well-drained soil had lower levels of elements compared with poorly drained soil. The correlations between the soil properties and elements varies with different elements. However, the difference between the andic and non-andic property is obvious. The andic soil could be related to lower concentrations of most of the elements, but non-andic soil relates to higher concentrations of these elements in pulp samples. For instance, the concentration of Ca (r = 0.58, p= 0.04) and Mn (r = 0.60, p = 0.03). The opposite effect of andic and non-andic properties further illustrates the role of soil characteristics in the difference in elemental content and further confirms the results of the PCA. D'Antone et al. (2017) found that volcanic soil influences the elements in plants, and that the characterisation of these differences caused by volcanic soil could help to identify the place of cultivation of grape varieties. A similar trend of correlations between growing conditions and elemental composition was observed for the peel samples. For example, rainfall also inhibited the content of Ba elements in peel samples (r = -0.63, p = 0.02), and significant opposite effects were observed in the andic and non-andic properties. These findings are also consistent with PCA results in Figure 2.3. As shown in the PCA plots, the reason could be due to the soil contents changing as a result of the volcanic activity north of the *Reventazon* river. Recent studies further explained the significant correlation between elemental compositions in soil and food materials. Taking Western European wines as an example, it is evident that the soil type has a big influence on the Mg and Ba contents in wine. In addition, climatic factors such as temperature could increase the Sr content in wines, which agree with the findings of this manuscript (Blotevogel et al., 2019). In our study, the

effects of soil features are also reflected in the elemental compositions of banana pulp and peel samples.

(A)



(B)



(C)

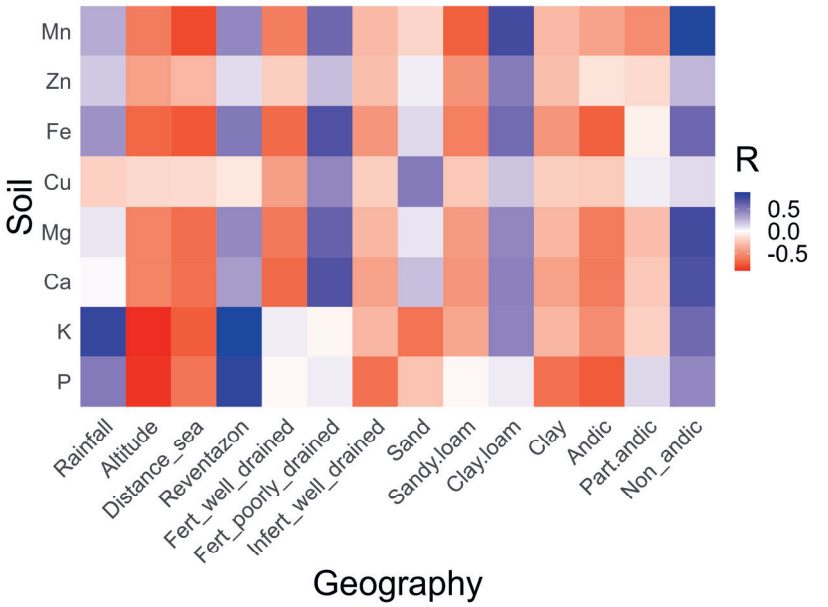


Figure 2.4. Pearson correlation analyses between the elemental compositions and growing conditions of the banana pulp samples (A), banana peel samples (B), and soil samples (C) collected from the different farms in Costa Rica. (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

The effects of growing conditions on soil elements are shown in Figure 2.4C. Rainfall was positively correlated with the K content in the total soil content (r = 0.78, p = 0.01), altitude showed a strong negative correlation with most of the elements in the soil such as P (r = -0.86, p = 0.003), K (r = -0.89, p = 0.001), and Fe (r = -0.69, p = 0.04). The correlations of andic and non-andic properties were opposite. The presence of non-andic soil increases the contents of P, K, Ca, Mg, Cu, Fe, Zn, and Mn in soil. Furthermore, andic soil shows the opposite effect for them, which further illustrates that elemental analysis can reflect changes in elemental contents resulting from volcanic activity. The distribution of elemental composition in soil from the sampling sites were different, which could be caused by the local growing conditions especially by the local soil properties.

2.4. Conclusions

The results verify that banana pulp, banana peel and the soil from different farms in Costa Rica have their own elemental and stable isotopic signatures. In effect, this research proved the potential of using stable isotopes and elemental analysis to discriminate the geographical origin of bananas. The elemental composition results revealed that the differences caused by volcanic activity to farm soil sources was an important factor contributing to the elemental differences of bananas. The combined effects of climate, soil, and precipitation were the main causes of differences in the carbon, nitrogen, and oxygen isotopes, while it also affects the extent to which bananas use mineral elements in the soil. The stable isotopes and elements such as Zn, Mg, Cu, Fe, Mn, and Al correlated with local soils and can be regarded as good indicators for the geographical origin using multivariate analyses. These findings are helpful to better understand the effects of growing conditions on the differences in banana compositions, while it also provides a mean of increasing the fruit's traceability by linking it to its origin. In future studies, more samples from different countries, as well as the use of different analytical techniques and chemometrics will be considered to expand the geographical identification of bananas and their interaction with growing conditions.

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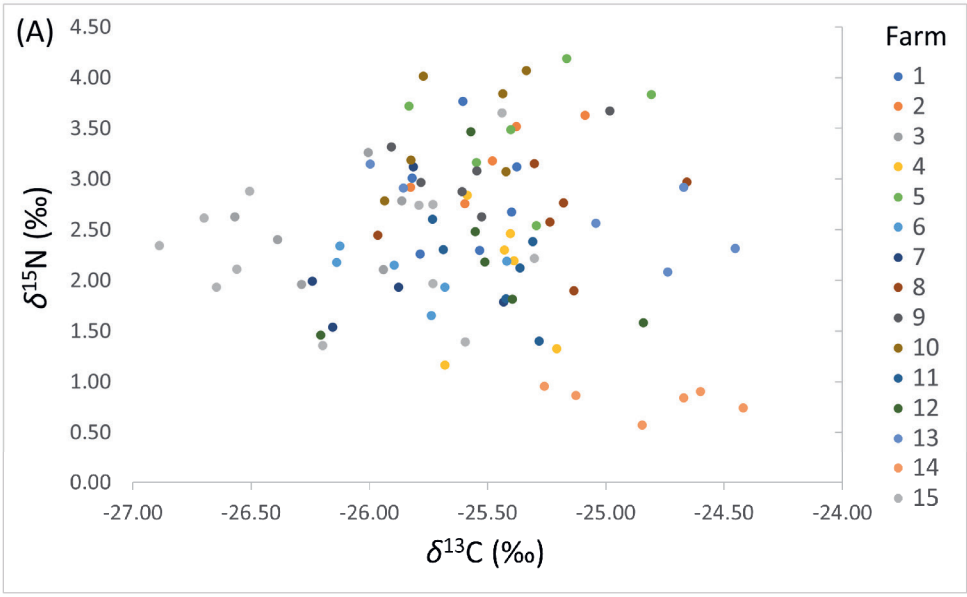
Appendix A.


(A)
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
δ15N (‰)
-27.00
-26.50
-26.00
-25.50
-25.00
-24.50
-24.00
δ13C (‰)
Farm
1
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3
4
5
6
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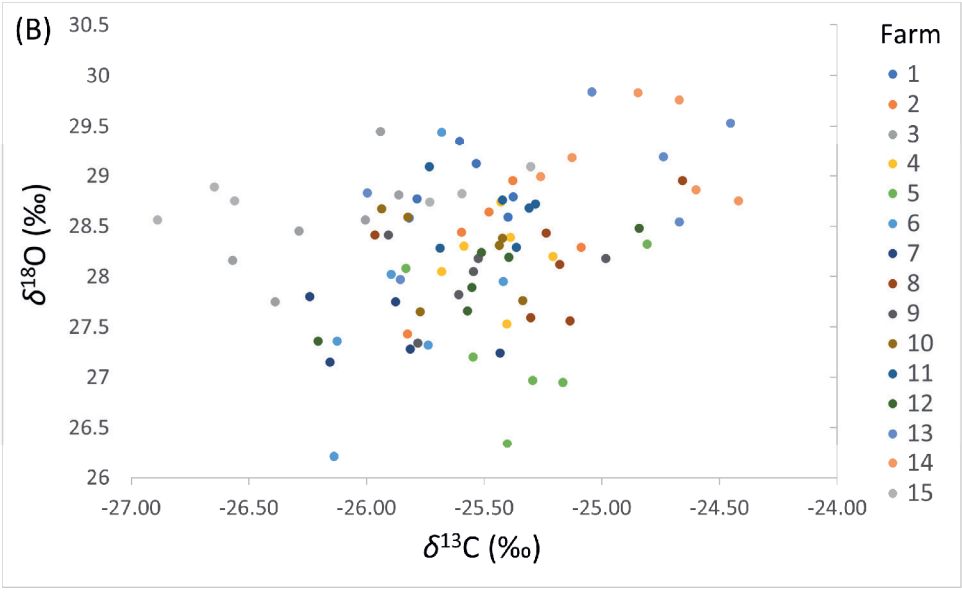
(B)
30.5
30
29.5
29
28.5
28
27.5
27
26.5
26
δ18O (‰)
-27.00
-26.50
-26.00
-25.50
-25.00
-24.50
-24.00
δ13C (‰)
Farm
1
2
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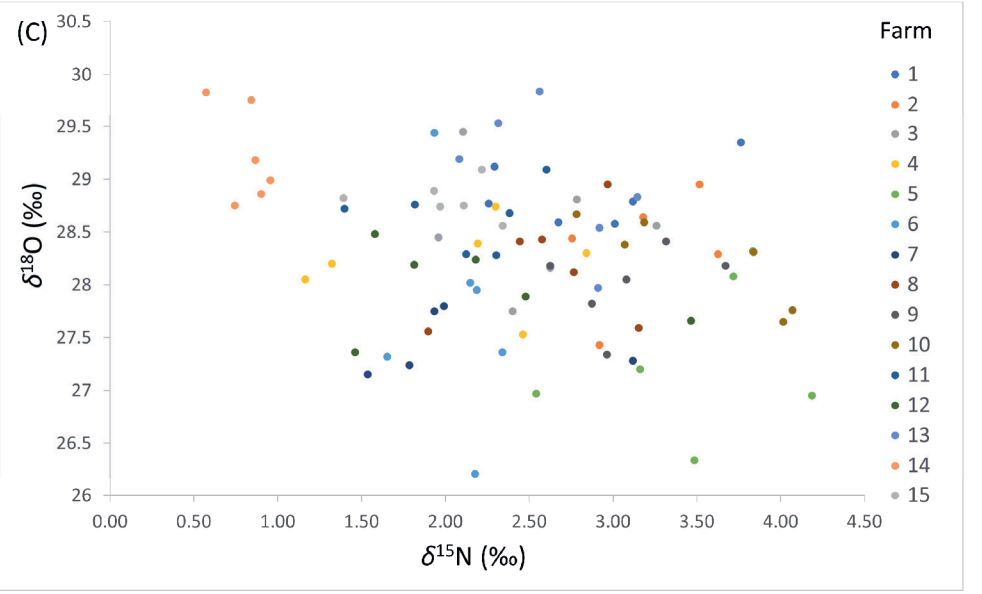

Figure A1. Scatter plot of the stable isotopic ratios (‰) of banana: $\delta^{13}C$ vs. $\delta^{15}N$ (A); $\delta^{13}C$ vs. $\delta^{18}O$ (B); $\delta^{18}O$ vs. $\delta^{15}N$ (C). (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.).

CHAPTER 3

Tracing the origin and exploring the relations between growing conditions and isotopic and elemental fingerprints of organic andconventional Cavendish bananas (*Musa* spp.)

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Wang, Z., Erasmus, S. W., & van Ruth, S. M. (2021). Preliminary study on tracing the origin and exploring the relations between growing conditions and isotopic and elemental fingerprints of organic and conventional Cavendish bananas (*Musa* spp.). *Foods, 10*(5), 1021.

Abstract

The stable isotopic ratios and elemental compositions of 120 banana samples, *Musa* spp. (AAA Group, Cavendish Subgroup) cultivar Williams, collected from six countries (Colombia, Costa Rica, Dominican Republic, Ecuador, Panama, Peru), were determined by isotope ratio mass spectrometry and inductively coupled plasma mass spectrometry. Growing conditions like altitude, temperature, rainfall, and production system (organic or conventional cultivation) were obtained from the sampling farms. Principal component analysis (PCA) revealed separation of the farms based on geographical origin and production system. The results showed a significant difference in the stable isotopic ratios ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$) and elemental compositions (Al, Ba, Cu, Fe, Mn, Mo, Ni, and Rb) of the pulp and peel samples. Furthermore, $\delta^{15}N$ was found to be a good marker for organically produced bananas. A correlation analysis was conducted to show the linkage of growing conditions and compositional attributes. The $\delta^{13}C$ of pulp and peel were mainly negatively correlated with the rainfall, while $\delta^{18}O$ was moderately positively (R values ~0.5) correlated with altitude and temperature. A moderate correlation was also found between temperature and elements such as Ba, Fe, Mn, Ni, and Sr in the pulp and peel samples. The PCA results and correlation analysis suggested that the differences of banana compositions were combined effects of geographical factors and production systems. Ultimately, the findings contribute towards understanding the compositional differences of bananas due to different growing conditions and production systems linked to a defined origin; thereby offering a tool to support the traceability of commercial fruits.

Key words

Banana, Elemental profiling, Geographical attribute, Stable isotopic fingerprinting

3.1. Introduction

Banana (*Musa* spp.) is one of the most representative fruits which are well-liked by consumers for their flavour and nutritional value. Generally, the cooking banana, also called plantain, is used as a staple food for millions of people in many countries, especially in developing economies, because it is an affordable source of energy (Oyeyinka & Afolayan, 2019). Another kind of popular banana is called the dessert banana, which is used as an everyday fruit because of its soft texture, sweet taste, and bioactive components (Aurore, Parfait, & Fahrasmane, 2009). The dessert banana is typically used as a food companion with products such as breakfast cereals, ice cream, and other desserts apart from just its raw consumption. In the current international market, the Cavendish variety are the most commercialized bananas and consumed world-wide (Dale et al., 2017). Cavendish bananas are rich in carbohydrates, vitamins, minerals, and dietary fibre, indicating that they have anti-oxidative and anti-ageing activities (Pereira & Maraschin, 2015).

In 2019 alone, around 21 million tons of bananas, excluding plantains, were exported from Ecuador, Colombia, Costa Rica, etc. Latin America and the Caribbean are the largest exporting regions, and the European Union (EU), United States, and China are the largest global importers (FAO, 2020). As the quality of the banana is highly dependent on climate and cultivation system, the label of origins is considered an important parameter for quality and price (Aprile, Caputo, & Nayga Jr, 2012). The production of organic bananas requires stricter management, such as forbidding the use of pesticides and chemical fertilisers (Bellamy, 2013). In the case of consumer preferences, the geographical origin and production system play a role in the purchase intent of consumers (Santeramo & Lamonaca, 2020). However, there are only a few papers about the authentication of the geographical origin and production system of bananas.

In recent years, several papers have been published concerning the geographical origin and organic production of agricultural products (Katerinopoulou, Kontogeorgos, Salmas, Patakas, & Ladavos, 2020; Shen, Zhao, Zhang, Huang, & Xie, 2021; Sun, Guo, & Wei, 2016). This is because food fraud and public health incidents occur frequently around the world. Food fraud, mainly known as economically motivated adulteration, is an ongoing problem (Robson, Dean, Haughey, & Elliott,

2021). Although the banana is not an extremely vulnerable target, authenticity research of bananas still has important value (van Ruth, Luning, Silvis, Yang, & Huisman, 2018). The banana is a staple food in many developing countries to ensure household food security. Meanwhile, the income from the banana industry is also important for approximately 400 million workers globally (FAO, 2020). In recent years, the production of bananas is facing many challenges, such as infectious diseases like *Fusarium wilt*, mislabelled organic bananas, sustainable production with less pesticides, etc. A reliable tracing system is helpful in banana chains to protect smallholder farmers, the retailer from unfair competition, and make sure the consumers get what they pay for (van Ruth, Huisman, & Luning, 2017). Previous studies have reported that the combined application of stable isotopes and elemental compositions could effectively identify the geographical origin of a variety of agricultural products such as grape wine (Bronzi et al., 2020), pork (Zhao et al., 2020), honey (Magdas et al., 2021), tea (Baskali Bouregaa et al., 2020), etc. Nevertheless, the measurement of stable isotopes could also reveal if the foods were produced by organic cultivation. The combination of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ could provide a sound solution for rice origin using chemometrics (Wang et al., 2020). For bell peppers, the linking of $\delta^{18}O$ in the sample and local water could indicate the geographical origin (de Rijke et al., 2016). Previous research also confirmed that elemental analysis of Mn, Cr, Sr, Ag, and Co could differentiate wines from different Romanian vineyards (Geana et al., 2013). In terms of bananas, there are limited papers about the geographical origin of bananas. One relevant study explored the impact of farming type, variety, and geographical origin on the bacterial community of bananas, and was aimed at discriminating organic bananas from conventional ones (Bigot et al., 2020). Most of the published research focused on the discrimination of geographical origin and production system, while only a few research studies aimed to explore the underlining reasons that foods have various composition differences according to different origins and production systems. There are already studies which reported that the lithological properties of origin could change the element contents of garlic (Choi, Bong, Park, & Lee, 2020), while latitudes had significant influences on the depleted $\delta^{2}H$ and $\delta^{18}O$ values of poultry (Rees et al., 2016). Chapter 2 revealed that the isotopic ratios and elemental profiles of bananas were highly related to climate, topography, and soil conditions in a single country (Chapter 2, Wang, Erasmus, Dekker, et al., 2020). To the best of our knowledge, only limited works have studied the relation of growing conditions and the composition of

bananas in different countries. In fact, most of the related studies focuses on ways to alter the growing conditions to increase the yield of bananas.

In this research chapter, the main stable isotope ratios and elemental compositions of banana samples from different origins were characterised with the aim to establish correlations among these fingerprints and the growing conditions of bananas. Banana samples, including pulp and peel, were collected from ten farms located in six different countries in South America. Besides the related growing conditions such as altitude, rainfall, and temperatures, some farms cultivated bananas according to an organic and some to a conventional production practice. The stable isotopic ratios and elements of pulp and peel from different countries were firstly characterised through isotope ratio mass spectrometry (IR-MS) and inductively coupled plasma mass spectrometry (ICP-MS). Furthermore, the correlation between stable isotopic and elemental fingerprints of organic/conventional bananas and their growing conditions was investigated. Due to the limitations in exploring the effects of external (environmental) factors such as harvest year and season together with the use of limited samples numbers, the paper was aimed to provide the feasibility study for the relations between growing conditions and isotopic and elemental fingerprints of bananas.

3.2. Materials and methods

3.2.1. Sample collection and growing conditions

Banana (*Musa* spp.) samples (AAA Group, Cavendish Subgroup) of the cultivar (cv.) Williams were collected in a 3-month period from February to April 2018 from ten farms located in six different countries namely, Colombia, Costa Rica, Dominican Republic, Ecuador, Panama, Peru. The locations of the farms, based on their Global Positioning System (GPS) coordinates, are shown in Figure 3.1. For each farm, twelve bunches from the top position of the banana trees were randomly selected. The bunches were then placed in clean polyethene bags, labelled with the sampling site location and transported in low temperature and dark conditions to Wageningen University & Research. Upon receipt of the bunches, two banana fingers were randomly selected from each bunch and combined as one research sample. The sampled bananas were then separated into pulp and peel, cut into slices, freeze-dried, and crushed to a fine

powder. Ultimately, 120 powdered banana pulp and peel samples were collected and stored at −20 °C until IR-MS and ICP-MS analyses. The growing conditions that include the geographical factors and production system data were also obtained from local farms. The overview of the collected banana samples, altitude, monthly mean temperature, annual rainfall, and production system data is shown in Table 3.1.

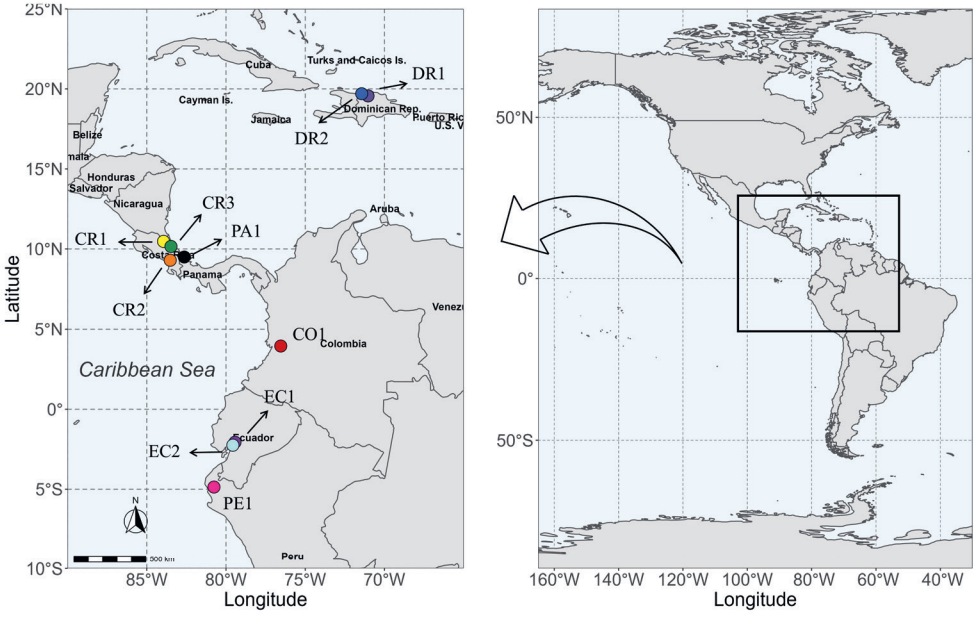


Figure 3.1. The sampling sites of the collected bananas. CO: Colombia; CR: Costa Rica; DR: Dominican Republic; EC: Ecuador; PA: Panama; PE: Peru; the numbers refer to the individual farms in a specific country. (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

Table 3.1. The banana pulp and peel samples collected from different countries and related growing conditions.

Country	Farm	Pulp (*n*)	Peel (*n*)	Altitude (m)	Monthly mean temperature (°C)	Annual rainfall (mm/Year)	Production system
Colombia	CO1	12	6	66	23.2	1837	Conventional
	CR1	12	6	726	23.4	2857	Conventional
Costa Rica	CR2	12	6	47	24.4	5014	Conventional
	CR3	12	6	24	26.3	1837	Conventional
Dominican	DR1	12	6	65	26.7	925	Organic
Republic	DR2	12	6	27	26.7	925	Organic
Ecuador	EC1	12	6	32	22.9	1511	Organic
	EC2	12	6	22	26.5	843	Conventional
Panama	PA1	12	6	20	19.7	3679	Conventional
Peru	PE1	12	6	40	24.1	200	Organic

3.2.2. Determining the stable isotope ratios

The three stable isotope ratios of carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$), and oxygen ($^{18}O/^{16}O$) of bananas were determined according to the protocol by Erasmus et al. (2016) with some modifications. The banana pulp and peel samples were analysed for $\delta^{13}C$ and $\delta^{15}N$ using an isotope ratio mass spectrometer (Flash 2000 in combination with Delta V advantage, Thermo Scientific, Waltham MA USA). The results were expressed as stable isotope ratios ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) against Vienna Pee Dee Belimnite (VPDB) for $\delta^{13}C$ and atmospheric AIR for $\delta^{15}N$. The oxygen isotope ratios were performed by a SerCon high-temperature elemental analyzer interfaced with a SerCon 20-20 IR-MS with SysCon electronics (Sercon, Cheshire, UK). The final delta unit ($\delta^{18}O$) was expressed as $^{18}O/^{16}O$ relative to international standards VSMOW2 (Vienna Standard Mean Ocean Water). For $\delta^{13}C$ and $\delta^{15}N$, the samples were weighed into tin capsules, while 4 × 6 mm silver cups were used for $\delta^{18}O$ analysis. To reduce the uncertainty and ensure the quality of the measurement, the international atomic energy agency (IAEA) standard samples (IAEA-600 for Carbon, Nitrogen, and IAEA-601 for Oxygen) were used. Every 12 samples were interspersed with a standard sample for calibration. All analyses were conducted in triplicate.

3.2.3. Determining the elemental compositions

The 11 kinds of elemental compositions (Al, Ba, Cr, Cu, Fe, Mn, Mo, Ni, Rb, Sr, and Zn) of banana pulp and peel were determined by ICP-MS (Herwig, Stephan, Panne, Pritzkow, & Vogl, 2011). The freeze-dried and fine homogeneous samples (pulp/peel) were digested using a MARS 6 microwave (CEM Corporation, Matthews NC, USA). About 0.8 g sample was accurately weighed into Teflon vessels. For digestion, 3 mL 70% nitric acid (HNO_3) and 1 mL 30% hydrogen peroxide (H_2O_2) were added to each vessel. Besides, another vessel only within HNO_3 and H_2O_2 was selected as a blank group. After digestion, the samples were transferred to tubes and filled up to 10 mL with Milli-Q water. Standard solutions were prepared from 1000 mg/L ICP-MS stock solutions (ICP-MS calibration standard, ULTRA Scientific, North Kingstown, Rhode Island). The multi-elemental analyses were performed using a NexION 300D ICP-MS (Perkin Elmer, Waltham, MA, USA). The analytical performance was verified by processing certified reference materials Lichen 482 (Sigma-Aldrich, Buchs, Switzerland), tuning solution A and B (Merck, Darmstadt, Germany). The concentrations of elemental composition in the samples were determined using external calibration curves and the rhodium was used as an internal standard.

3.2.4. Statistical analysis

The compositional data were statistically analysed using a one-way analysis of variance (ANOVA) and Tukey's test (multiple-range) to assess the impact of the geographical factors and production system of bananas, as a single categorical factor, on the different stable isotopic ratios and elemental compositions. Means with *p* values below 0.05 were significantly different (Yan, Oey, van Leeuwen, & van Ruth, 2018). The multivariate characterisation of the samples concerning stable isotopic ratios and elements, and growing conditions were performed by multiple variable regression and principal component analysis (PCA) (Vera, Jiménez-Carvelo, Cuadros-Rodríguez, Ruisánchez, & Callao, 2019). The stable isotopic ratios and elemental compositions were pre-processed using a log transformation before PCA analysis was performed. Pearson correlation coefficients (r) were calculated to evaluate the correlation between growing conditions and banana compositions (N. Liu et al., 2020). Data analyses and the visualizations were conducted using R 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

3.3. Results and discussion

3.3.1. Summary of the growing conditions of banana farms

The geographical factors (altitude, monthly mean temperature, and annual rainfall) and production system (organic and conventional cultivation) were regarded as growing conditions of the banana farms. In total, ten farms from six different countries were selected as sampling sites due to their differences in growing conditions between the different farms (Figure 3.1). Table 3.1 shows the six conventional farms (CO1, CR1, CR2, CR3, EC2, PA1) and four organic farms (DR1, DR2, EC1, PE1). The farms were specifically selected to explore the effects of geographical factors and production system. For example, the farms with a conventional production system, but from different countries, could be used to compare the effects of geographical origins. Whereas the farms with different production systems, but from the same country, allow determination of the effect of production systems. Figure 3.2 indicates the differences in geographical factors such as altitude, temperature, and rainfall due to the different locations. The ten farms occupied different spatial locations on the scatterplot composed of altitude, temperature, and rainfall. For example, the farm CR2 from Costa Rica had the highest annual rainfall of 5014 mm/year and an altitude of 47 m. Therefore, it grouped separately from Farm PE1 with a rainfall of 200 mm/year and an altitude of 40 m. However, farms EC2, DR1, and DR2 grouped closely together as they share similar geographical factors.

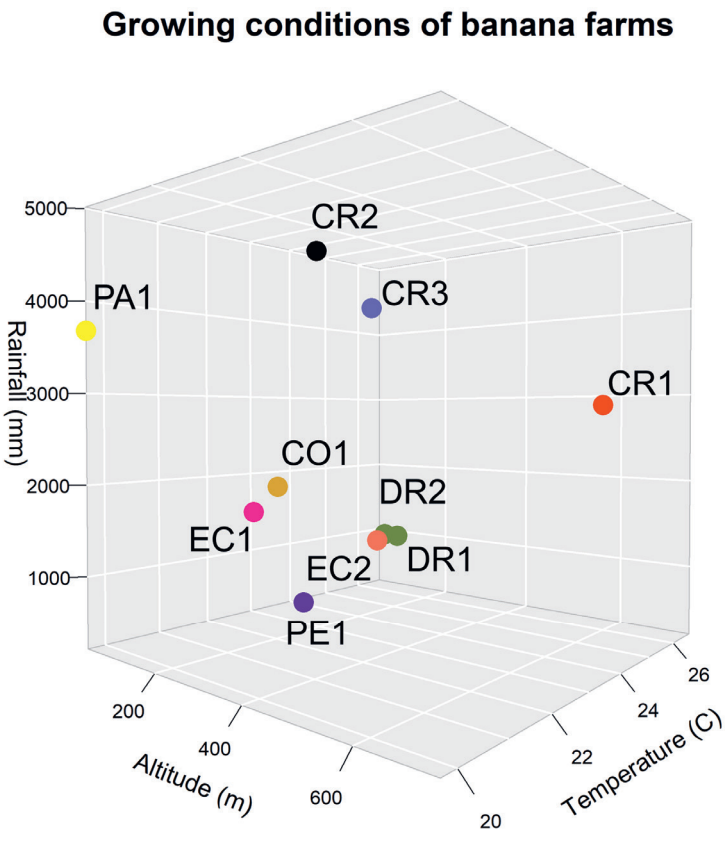


Figure 3.2. Differences in geographical factors of banana farms from Colombia (CO), Costa Rica (CR), Dominican Republic (DR), Ecuador (EC), Panama (PA), and Peru (PE). The numbers refer to individual farms in a country. (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

3.3.2. Differences of stable isotopes between bananas from different growing conditions

For the banana pulp samples, as shown in Table 3.2, the three stable isotopes ratios ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$) of bananas from different countries in Latin America were compared. For all ten farms, the $\delta^{13}C$ of pulp samples ranged from −23.8‰ (CR2) to −22.6‰ (PE1). The $\delta^{13}C$ value for PE1 was significantly different to the carbon isotope ratio of CR3, EC2, and PA1. For the $\delta^{15}N$ value, the results of DR2 (6.3 ± 1.3‰) was markedly higher than the ratios for the other farms. In fact, the $\delta^{15}N$ values for the

conventional farms (CR1, CR3, EC2, and PA1) were significantly lower than most of the organic farms such as DR1, DR2, EC1, and PE1. The stable isotope ratios of oxygen were the highest for CR1 (31.9 ± 0.7‰), while EC2 had the lowest value at 28.4 ± 0.5‰.

The stable isotope ratios of the peel samples were consistent with that of the pulp samples. Table 3.3 shows that the values of $\delta^{13}C$ ranged from −25.6‰ (DR2) to −23.6‰ (DR1 and PE1). The variation among the $\delta^{15}N$ values of the samples was greater than that of the $\delta^{13}C$ values. The nitrogen ratios of the organic farms were significantly higher than the conventional farms, such as 8.0 ± 0.4‰ for DR2 and 0.8 ± 0.6‰ for PA1. For the $\delta^{18}O$ result, the highest value was reported for DR1 (29.9 ± 0.3‰), while the lowest was 25.2 ± 0.5‰ (EC2). The values from different farms changed according to related growing conditions ($p < 0.05$). Regarding method precision, the standard deviations of repeated pulp samples ($n = 12$) of 10 groups ranged from 0.2–0.8‰ for $\delta^{13}C$, 0.1–1.5‰ for $\delta^{15}N$ and 0.3–1.1‰ for $\delta^{18}O$. For the peel samples, the standard deviations of repeated measurements ($n = 6$) ranged from 0.2–0.8‰ for $\delta^{13}C$, 0.2–0.6‰ for $\delta^{15}N$ and 0.3–1.3‰ for $\delta^{18}O$.

Table 3.2. The analysis of variance (ANOVA) of the stable isotope ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$, and $^{18}O/^{16}O$) and elemental compositions (mg/kg) of banana pulp samples (n = 12).

Farm	$\delta^{13}C$	$\delta^{15}N$	$\delta^{18}O$	Al	Ba	Cu	Fe	Mn	Mo	Ni	Rb	Sr	Zn
CO1	$-23.0^{ab} \pm 0.8$	$2.9^{b} \pm 0.8$	$30.9^{bc} \pm 0.5$	$0.8^{bcd} \pm 0.2$	$1.6^{a} \pm 0.2$	$3.6^{cd} \pm 0.5$	$11.5^{b} \pm 1.3$	$15.7^{b} \pm 3.8$	$0.1^{cd} \pm 0.1$	$0.3^{a} \pm 0.1$	$1.9^{c} \pm 0.3$	$2.6^{b} \pm 0.4$	$6.1^{bcd} \pm 1.1$
CR1	$-23.3^{ac} \pm 0.5$	$-0.9^{f} \pm 0.9$	$31.9^{a} \pm 0.7$	$0.9^{ac} \pm 0.3$	$0.5^{b} \pm 0.1$	$3.9^{bcd} \pm 0.6$	$10.7^{b} \pm 0.9$	$21.9^{a} \pm 5.5$	$0.0^{d} \pm 0.0$	$0.3^{a} \pm 0.1$	$1.3^{c} \pm 0.3$	$2.0^{bc} \pm 0.5$	$5.2^{de} \pm 0.8$
CR2	$-23.8^{ac} \pm 0.6$	$1.1^{cd} \pm 1.5$	$31.4^{ab} \pm 0.6$	$1.2^{a} \pm 0.5$	$1.6^{a} \pm 0.3$	$4.5^{ab} \pm 0.4$	$12.7^{a} \pm 0.5$	$12.9^{bc} \pm 1.9$	$0.2^{b} \pm 0.0$	$0.1^{cd} \pm 0.0$	$9.0^{b} \pm 1.1$	$2.5^{b} \pm 0.6$	$5.9^{bce} \pm 1.2$
CR3	$-23.5^{bc} \pm 0.4$	$-0.5^{ef} \pm 0.6$	$31.2^{ac} \pm 0.4$	$0.9^{bc} \pm 0.1$	$0.4^{bc} \pm 0.0$	$3.7^{cd} \pm 0.2$	$10.7^{b} \pm 0.6$	$9.2^{ce} \pm 2.9$	$0.2^{b} \pm 0.1$	$0.2^{cd} \pm 0.0$	$7.0^{b} \pm 2.0$	$1.9^{bc} \pm 0.2$	$5.9^{bce} \pm 0.5$
DR1	$-23.3^{ac} \pm 0.5$	$1.9^{cd} \pm 0.4$	$30.5^{c} \pm 0.3$	$0.6^{cd} \pm 0.2$	$0.3^{cd} \pm 0.0$	$4.2^{ac} \pm 0.7$	$8.8^{c} \pm 0.7$	$5.2^{f} \pm 1.7$	$0.3^{a} \pm 0.1$	$0.1^{cd} \pm 0.0$	$1.9^{c} \pm 0.8$	$3.3^{a} \pm 0.6$	$7.0^{ab} \pm 0.6$
DR2	$-23.2^{ac} \pm 0.6$	$6.3^{a} \pm 1.3$	$30.9^{bc} \pm 1.1$	$0.6^{cd} \pm 0.3$	$0.5^{bc} \pm 0.2$	$4.5^{bd} \pm 0.8$	$9.3^{c} \pm 0.9$	$7.6^{ef} \pm 2.5$	$0.2^{b} \pm 0.1$	$0.1^{d} \pm 0.0$	$2.9^{c} \pm 1.8$	$3.5^{a} \pm 0.6$	$7.1^{a} \pm 1.1$
EC1	$-23.1^{ac} \pm 0.6$	$1.5^{ce} \pm 0.6$	$29.1^{d} \pm 0.7$	$0.7^{cd} \pm 0.2$	$0.2^{d} \pm 0.1$	$2.8^{e} \pm 0.2$	$8.6^{c} \pm 0.5$	$5.6^{ef} \pm 0.8$	$0.1^{d} \pm 0.0$	$0.2^{bc} \pm 0.0$	$7.3^{b} \pm 2.6$	$1.2^{de} \pm 0.4$	$6.0^{bce} \pm 0.4$
EC2	$-23.6^{bc} \pm 0.2$	$-0.4^{ef} \pm 0.2$	$28.4^{d} \pm 0.5$	$0.4^{d} \pm 0.1$	$0.3^{bd} \pm 0.0$	$3.6^{cd} \pm 0.4$	$8.8^{c} \pm 0.5$	$5.3^{ef} \pm 1.2$	$0.2^{bc} \pm 0.0$	$0.2^{b} \pm 0.0$	$1.8^{c} \pm 0.4$	$1.0^{e} \pm 0.1$	$6.0^{bcd} \pm 0.3$
PA1	$-23.4^{bc} \pm 0.2$	$0.0^{def} \pm 0.1$	$31.0^{ac} \pm 0.4$	$0.8^{bc} \pm 0.2$	$0.5^{bd} \pm 0.1$	$4.7^{a} \pm 0.4$	$12.7^{a} \pm 0.8$	$11.6^{cd} \pm 4.2$	$0.2^{b} \pm 0.0$	$0.1^{d} \pm 0.0$	$3.4^{c} \pm 1.5$	$2.0^{bc} \pm 0.4$	$6.8^{ac} \pm 0.5$
PE1	$-22.6^{a} \pm 0.3$	$1.7^{c} \pm 0.3$	$31.5^{ab} \pm 0.8$	$1.1^{ab} \pm 0.2$	$0.4^{bc} \pm 0.1$	$3.4^{de} \pm 0.2$	$8.7^{c} \pm 0.7$	$8.0^{def} \pm 2.0$	$0.2^{bc} \pm 0.0$	$0.1^{d} \pm 0.0$	$12.1^{a} \pm 5.2$	$1.7^{cd} \pm 0.2$	$5.0^{e} \pm 0.3$

$^{a–f}$ Different mean values in the same column with different superscript letters are significantly different ($p < 0.05$) according to Tukey's significant difference test; the Cr was not presented because of values lower than limit of detection.

There are several studies reporting that the $\delta^{15}N$ value could be used to determine if a food product were produced organically (Laursen, Schjoerring, Kelly, & Husted, 2014). It is well-known that the main type of nitrogen isotope in synthesized fertilisers is isotope ^{14}N, while the proportion of the heavier isotope ^{15}N is much higher than ^{14}N in organic fertilisers (Laursen et al., 2013). This is because the conventional fertilisers are chemically synthesized from N_2 in the air under high temperature and high pressure, the content of ^{15}N in chemical nitrogen fertiliser is equivalent to that in the atmosphere. However, organic fertilisers are derived from animal matter, animal manure, and vegetable matter, therefore, the stable isotope ^{15}N is continuously enriched in plant and animals due to bioconcentration effects. Due to the enrichment of ^{15}N, the abundance of ^{15}N in plants or animals is significantly higher than that in air. Therefore, the abundance of ^{15}N in organic fertilisers is significantly higher than that in chemical fertilisers (Camin, Bontempo, Perini, & Piasentier, 2016). In line with the literature, the pulp and peel samples from organic farms such as DR1, DR2, EC1, and PE1 had generally higher $\delta^{15}N$ ($^{15}N/^{14}N$) values than the samples from some of the other conventional farms. The highest $\delta^{15}N$ was 6.3‰ and 8.0‰ in pulp and peel samples of farm DR2, respectively. Most of the conventional farms were reported having $\delta^{15}N$ values lower than 1.0‰ except for CO1 with a nitrogen isotope ratio of 2.9‰ for pulp and 3.8‰ for the peel samples. This could be due to the fact that some farms choose to apply chemical and organic fertilisers simultaneously to improve soil vitality and agricultural sustainability (Mustaffa & Kumar, 2012). Therefore, the application of organic fertiliser could also improve the proportion of the stable isotope ^{15}N in the conventionally produced bananas of farm CO1.

Table 3.3. The analysis of variance (ANOVA) of stable isotopes ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$, and $^{18}O/^{16}O$) and elemental compositions (mg/kg) of banana peel samples (n = 6).

Farm	$\delta^{13}C$	$\delta^{15}N$	$\delta^{18}O$	Al	Ba	Cr	Cu	Fe	Mn	Mo	Ni	Rb	Sr	Zn
CO1	$-25.0^{bd} \pm 0.8$	$3.8^{b} \pm 0.3$	$25.7^{cd} \pm 0.3$	$24.3^{bc} \pm 2.6$	$8.7^{b} \pm 1.1$	$0.1^{ab} \pm 0.0$	$5.1^{ab} \pm 0.6$	$24.8^{a} \pm 9.3$	$43.2^{bcd} \pm 7.8$	$0.2^{bc} \pm 0.1$	$0.4^{a} \pm 0.1$	$3.1^{d} \pm 0.7$	$22.0^{c} \pm 3.5$	$17.8^{ab} \pm 1.7$
CR1	$-24.7^{bd} \pm 0.5$	$-1.1^{f} \pm 0.5$	$28.0^{b} \pm 1.0$	$23.5^{cd} \pm 7.5$	$3.6^{cd} \pm 0.6$	$0.1^{ab} \pm 0.1$	$4.0^{cd} \pm 0.3$	$19.6^{ac} \pm 1.4$	$82.2^{a} \pm 15.6$	$0.1^{d} \pm 0.0$	$0.3^{ab} \pm 0.1$	$6.0^{d} \pm 4.7$	$20.8^{cd} \pm 3.1$	$17.8^{ab} \pm 2.0$
CR2	$-25.0^{bd} \pm 0.3$	$0.5^{df} \pm 0.3$	$27.9^{b} \pm 1.3$	$24.7^{bc} \pm 5.9$	$10.6^{a} \pm 1.5$	$0.1^{ab} \pm 0.0$	$5.8^{a} \pm 0.7$	$21.8^{ab} \pm 1.2$	$61.7^{ab} \pm 26.9$	$0.2^{bc} \pm 0.0$	$0.2^{b} \pm 0.0$	$24.3^{bc} \pm 7.2$	$22.3^{c} \pm 8.5$	$15.7^{ab} \pm 1.9$
CR3	$-24.6^{bc} \pm 0.3$	$-0.3^{ef} \pm 0.2$	$28.2^{b} \pm 0.9$	$29.1^{bc} \pm 13.6$	$3.4^{cd} \pm 1.4$	$0.0^{b} \pm 0.0$	$4.5^{bc} \pm 0.8$	$16.2^{bcd} \pm 4.7$	$35.0^{ce} \pm 11.3$	$0.2^{bc} \pm 0.0$	$0.2^{c} \pm 0.1$	$16.4^{bd} \pm 3.9$	$21.8^{c} \pm 4.4$	$16.5^{ab} \pm 1.6$
DR1	$-23.6^{a} \pm 0.5$	$2.6^{c} \pm 0.4$	$29.8^{a} \pm 0.3$	$23.5^{cd} \pm 4.8$	$2.4^{cd} \pm 0.3$	$0.1^{ab} \pm 0.0$	$3.7^{cd} \pm 0.6$	$19.9^{ac} \pm 2.5$	$18.0^{ce} \pm 6.9$	$0.5^{a} \pm 0.1$	$0.2^{c} \pm 0.0$	$8.8^{cd} \pm 5.3$	$41.5^{a} \pm 4.6$	$19.0^{a} \pm 2.5$
DR2	$-25.6^{d} \pm 0.5$	$8.0^{a} \pm 0.4$	$27.2^{bc} \pm 1.3$	$5.4^{e} \pm 1.4$	$3.0^{cd} \pm 0.1$	$0.1^{a} \pm 0.0$	$3.4^{d} \pm 0.4$	$20.5^{ac} \pm 2.4$	$31.7^{ce} \pm 2.5$	$0.3^{b} \pm 0.0$	$0.2^{c} \pm 0.0$	$11.8^{cd} \pm 5.3$	$33.7^{b} \pm 1.9$	$15.3^{ab} \pm 2.7$
EC1	$-24.4^{ab} \pm 0.5$	$1.5^{cde} \pm 0.5$	$26.7^{bd} \pm 0.7$	$11.1^{de} \pm 2.3$	$1.8^{d} \pm 0.4$	$0.1^{ab} \pm 0.0$	$3.6^{cd} \pm 0.4$	$16.5^{bcd} \pm 1.0$	$28.0^{ce} \pm 3.6$	$0.1^{cd} \pm 0.0$	$0.3^{bc} \pm 0.0$	$28.7^{b} \pm 5.2$	$13.3^{de} \pm 2.4$	$15.5^{ab} \pm 1.4$
EC2	$-24.9^{bd} \pm 0.3$	$0.5^{df} \pm 0.2$	$25.2^{d} \pm 0.5$	$21.3^{cd} \pm 4.3$	$2.1^{cd} \pm 0.1$	$0.1^{ab} \pm 0.0$	$4.2^{bd} \pm 0.3$	$16.3^{bcd} \pm 1.4$	$21.2^{de} \pm 7.0$	$0.2^{bc} \pm 0.0$	$0.3^{bc} \pm 0.1$	$10.8^{cd} \pm 7.6$	$10.5^{e} \pm 2.1$	$14.3^{b} \pm 1.2$
PA1	$-25.3^{cd} \pm 0.3$	$0.8^{cde} \pm 0.6$	$28.4^{ab} \pm 0.8$	$19.8^{cd} \pm 1.7$	$2.6^{cd} \pm 0.6$	$0.0^{b} \pm 0.0$	$5.6^{a} \pm 0.5$	$13.7^{cd} \pm 0.8$	$45.8^{bc} \pm 18.7$	$0.2^{bc} \pm 0.0$	n.d.	$11.9^{cd} \pm 6.0$	$23.5^{c} \pm 3.8$	$19.0^{a} \pm 1.9$
PE1	$-23.6^{a} \pm 0.2$	$1.9^{cd} \pm 0.5$	$27.4^{b} \pm 0.8$	$37.3^{b} \pm 11.7$	$2.9^{cd} \pm 0.2$	$0.1^{ab} \pm 0.0$	$4.4^{bc} \pm 0.5$	$12.2^{d} \pm 1.2$	$34.0^{ce} \pm 4.5$	$0.2^{b} \pm 0.0$	$0.2^{c} \pm 0.1$	$47.7^{a} \pm 20.5$	$17.0^{ce} \pm 1.8$	$18.2^{a} \pm 1.9$

[a–f] Different mean values in the same column with different superscript letters are significantly different ($p < 0.05$) according to Tukey's significant difference test; n.d.: none detected.

3.3.3. Differences of elemental compositions between bananas from different growing conditions

More than 20 types of minerals were detected in the bananas by ICP-MS. In total, 10 elements in pulp and 11 elements in peel samples were reported higher than the limit of detection and hence, only these were selected to compare the effects of geographical factors (altitude, monthly mean temperature, and annual rainfall) and production system (organic and conventional cultivation). Regarding method precision, the relative standard deviations of pulp samples among all groups for elements, Fe were less than 10%, Cu, Ni, Sr, and Zn were less than 20% and Al, Ba, Mn, Mo, Rb were less than 30%. In the peel samples, slightly higher standard deviations were reported, with Ba, Cu, Fe, Mo, Sr, Zn less than 20%, and Al, Cr, Mn, Ni, Rb were ranged from 20% to 40%. The concentrations of elements for the banana pulp and peel samples obtained from the different countries are listed in Tables 3.2 and 3.3 and Tables S3.1 and S3.2 in Appendix B. For the pulp samples, 10 elements (Al, Ba, Cu, Fe, Mn, Mo, Ni, Rb, Sr, and Zn) were detected in the samples. The mean concentration of the elements was significantly different among farms. The concentration of Mn was reported as 21.9 mg/kg for CR1, which was higher ($p < 0.05$) than the concentration for DR1 (5.2 mg/kg). The pulp samples from the organic farms, DR1 and DR2, contained a significantly higher concentration of Zn. For the peel samples, 11 elements (Al, Ba, Cr, Cu, Fe, Mn, Mo, Ni, Rb, Sr, and Zn) were obtained and were significantly different according to geographical factors and the production system. The elemental profiles of the banana peel samples were quite different to that of the pulp samples; with the concentration of Al, Fe, Mn, Sr, and Zn being higher in the peel samples. The organic farm, PE1, had a notably lower concentration of Fe when compared to other conventional farms ($p < 0.05$). Most of the farms have significantly different concentrations of elements in the peel samples, but there were still a few farms that did not show any significant differences in some elements ($p > 0.05$). As seen in Tables 3.2 and 3.3, the concentrations of the elements of the pulp and peel samples were significantly different among most of the farms. The results were consistent with the findings of a recent paper about the elemental compositions of bananas (Happi Emaga, Andrianaivo, Wathelet, Tchango, & Paquot, 2007). Hardisson et al. (2001) detected Fe, Cu, Zn, Mn, and B in the banana pulp of *Musa* AAA bananas, the same variety used in the current study, which is in line with the current results. As reported in comparative

research of eight banana cultivars, the trace elements such as Zn, Fe, and Mn were also detected in the peel samples (Sulaiman et al., 2011).

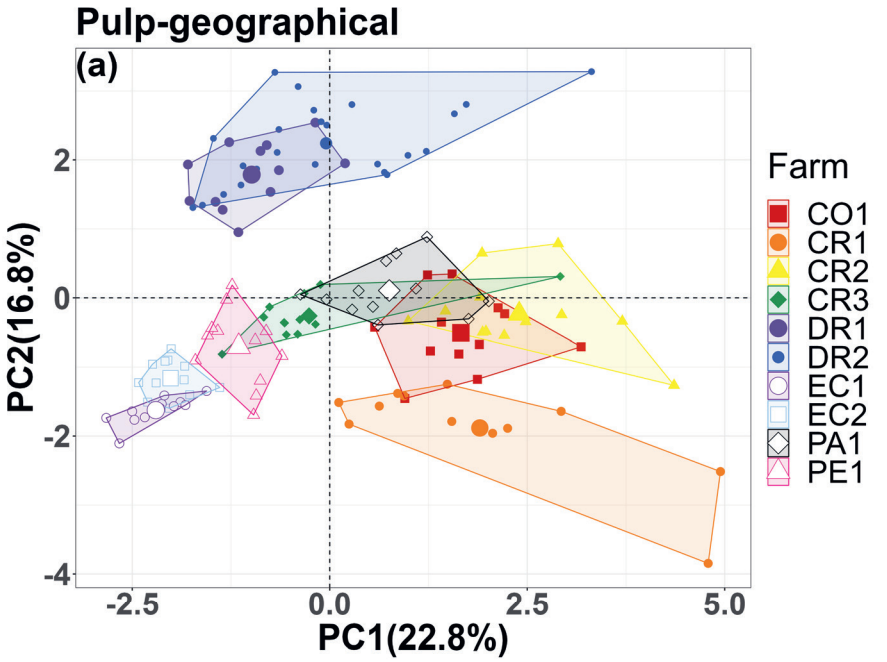
The current study systematically described and compared the trace elements of banana pulp and peel from different growing conditions. The ANOVA results indicates significant differences of elements for most of pulp and peel samples. For example, for the pulp samples, the farm DR2 showed the lowest concentration of Al at 5.4 mg/kg, significantly lower than farm DR1 at 23.5 mg/kg, even though both farms were located in the same country. Therefore, it is vital to also consider the geographical (or external) factors as opposed to just the “country border” when studies on the geographical origin of foods are performed. For example, the two geographically distant farms, DR2 (organic) and EC2 (conventional), have similar geographical factors in relation to altitude, temperature, and rainfall; the scatter plot (Figure 3.2) showed that the space location of both farms were almost coincident. Likewise, the concentration of most of the elements such as Ba, Cr, Cu, Fe, Mn, Mo, Ni, Rb, and Zn of the peel samples were not significantly different, although these two farms have different production systems. Hence, there are likely other external factors that have a greater influence on the variation in elemental compositions of bananas.

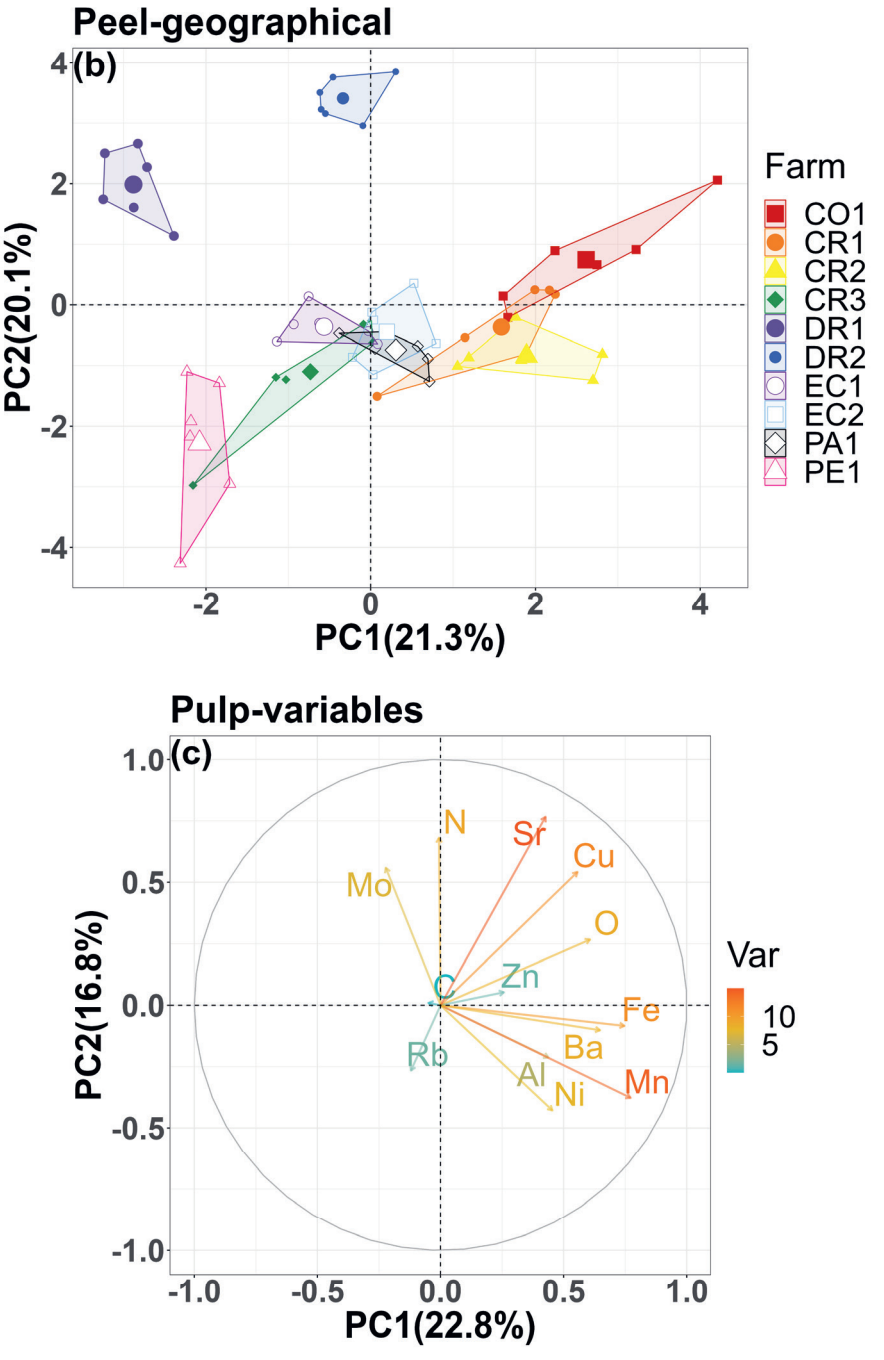
3.3.4. Principal component analysis of bananas between the different growing conditions

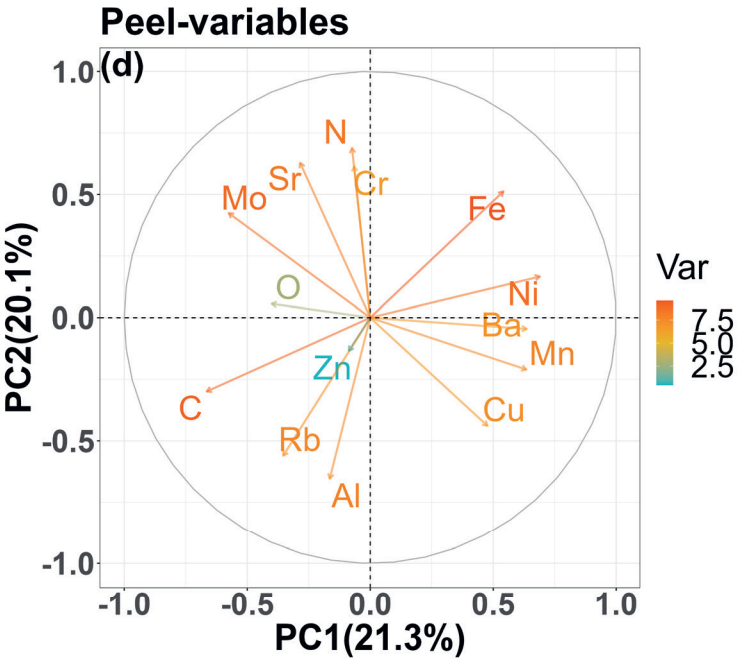
To explore the effects of growing conditions on bananas compositions, the IR-MS and ICP-MS data sets were subjected to multivariate data analysis. The PCA was used to show the distribution and groupings of the pulp and peel samples (Figure 3.3). The PCA plot shows that most of the farms could be separated according to differences in the stable isotopic ratios and elemental compositions of the samples. For the pulp and peel samples, PC1 and PC2 explained 39.6% and 41.4%, respectively, of the total variance. Some farms from the same countries, like CR1, CR2, and CR3 from Costa Rica and DR1, DR2 from the Dominican Republic, overlapped in the PCA plots. Figure 3.3a,e shows the farm distribution according to different geographical factors and production system, respectively. DR1 and DR2 were clearly separated from the other farms (Figure 3.3a). The differences in the concentrations of Mo, N, Sr, Rb, and Ni mainly contributed to the farms' distributions at geographical level. The effects of production system showed that the variables δ^{15}N, Mo, and Sr were characteristics of

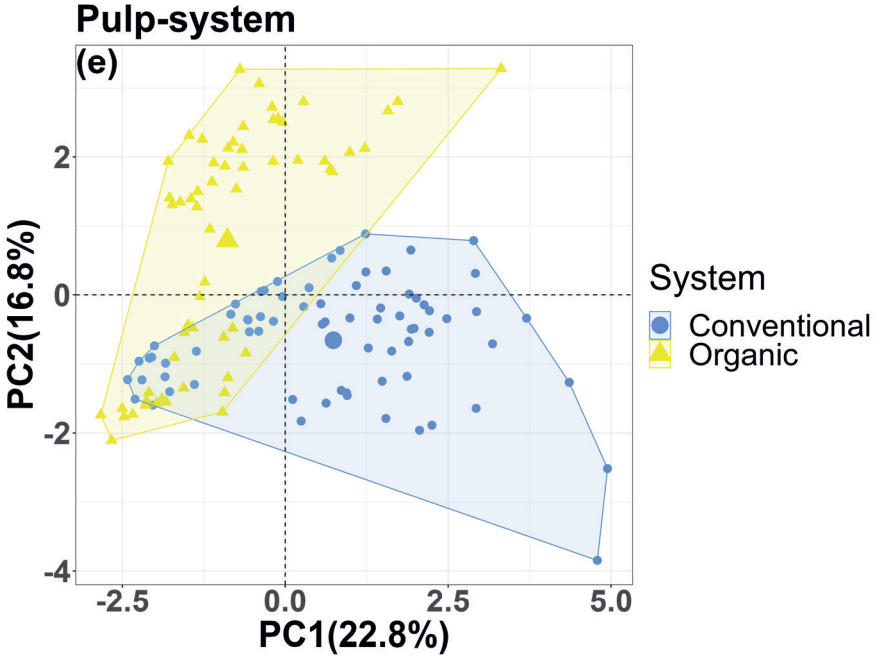
organic farms, while Mn, Ni, Al, Ba, and Fe were characteristics of conventional farms for the pulp samples (Figure 3.3e).

The stable isotope ratios and concentration of elements of the peel samples resulted in a different distribution of farms compared to the pulp samples. For instance, the concentration of Sr and Cu show a greater contribution to the discrimination of the peel samples based on geographical origin and production system. As seen in Figure 3.3b, the peel samples from DR1 and DR2 were grouped separately from samples from Central and South America mainland, with only a slight overlap seen between the different farms. Whereas Figure 3.3b show that the internal variation between peel samples within each farm is lower than their variation in pulp samples, Figure 3d shows that the variables $\delta^{15}N$, Mo, Sr, and Cr were characteristics of organic farms, while Ni, Ba, Mn, Cu, Al, Rb, and Zn were characteristics of conventional farms for the peel samples.




Peel-geographical
(b)
PC2(20.1%)
PC1(21.3%)
Farm
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Pulp-variables
(c)
PC2(16.8%)
PC1(22.8%)
Var
10
5
N
Sr
Cu
Mo
O
Zn
C
Fe
Rb
Ba
Al
Ni
Mn


Peel-variables
(d)
1.0
0.5
0.0
-0.5
-1.0
PC2(20.1%)
N
Sr
Cr
Mo
Fe
O
Ni
Ba
Mn
Zn
C
Cu
Rb
Al
-1.0
-0.5
0.0
0.5
1.0
PC1(21.3%)
Var
7.5
5.0
2.5


Pulp-system
(e)
2
0
-2
-4
PC2(16.8%)
-2.5
0.0
2.5
5.0
PC1(22.8%)
System
Conventional
Organic

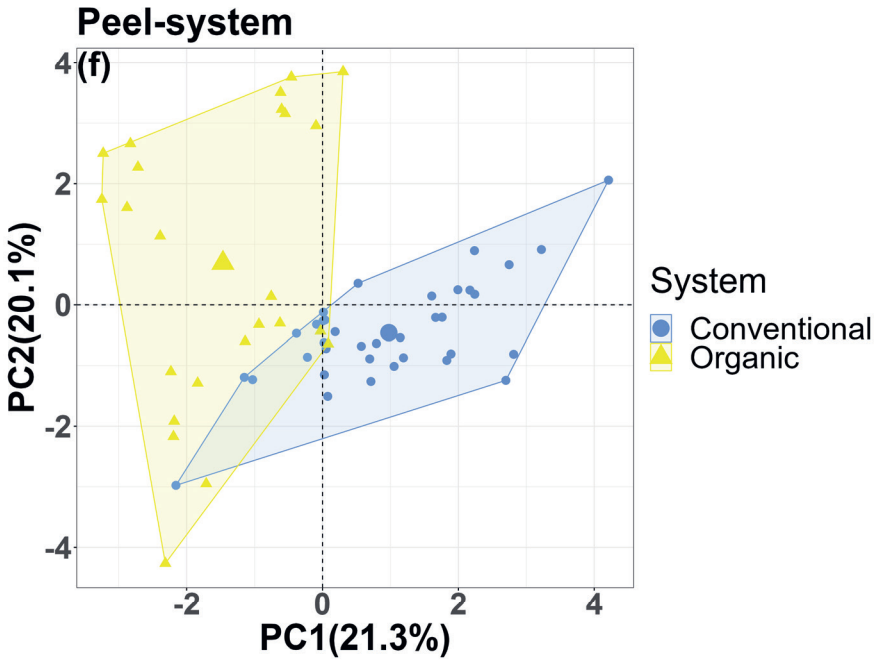


Figure 3.3. The principal component analysis (PCA) of banana pulp (*n* = 12) and peel (*n* = 6) samples according to stable isotopic ratios and elemental compositions. (**a**) PCA plot showing scores for the pulp samples from the different geographical origins and loadings for the variables. Explained variance: PC1 = 22.8%, PC2 = 16.8%; (**b**) PCA plot showing scores for the peel samples from the different production systems and loadings for the variables. Explained variance: PC1 = 21.3%, PC2 = 20.1%; (**c**) the loading plot of stable isotopic ratios and elemental compositions for banana pulp samples; (**d**) the loading plot of stable isotopic ratios and elemental compositions for banana peel samples; (**e**) PCA plot showing scores for the pulp samples from the different geographical origins and loadings for the variables. Explained variance: PC1 = 22.8%, PC2 = 16.8%; (**f**) PCA plot showing scores for the peel samples from the different production systems and loadings for the variables. Explained variance: PC1 = 21.3%, PC2 = 20.1%.CO: Colombia; CR: Costa Rica; DR: Dominican Republic; EC: Ecuador; PA: Panama; PE: Peru. (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

There is considerable evidence that the application of stable isotopes and elements is of great interest for tracing the geographical origin and production system

of many food products, such as tea (Peng et al., 2019), maca (He et al., 2020), orange (Rapisarda et al., 2010), etc. The use of multi-isotopes (i.e., $\delta^{13}C$, $\delta^{15}N$) and multi-elements (e.g., Na, Fe, Zn, and K) have been reported as reliable tools in authenticity testing of organically grown rice (Liu et al., 2020). In this research chapter, the combination of stable isotopic ratios and elemental compositions were employed for the visualization of multifactorial analysis. The PCA loadings and the contribution of variances are shown in Figure 3.3c,d. A clear separation was identified between organic and conventional production for the pulp and peel samples. The most important variables driving the separation between groups were $\delta^{13}C$, Mo, N, Fe, Ba, Mn, and Ni for the pulp samples, and $\delta^{18}O$, $\delta^{15}N$, Se, Mo, Cu, Al, Mn, and Ba for the peel samples. The different contribution of elements in the pulp and peel sample could be because the distribution of minerals in the different parts of the fruits were different. For the results on the geographical origin of the bananas (Figure 3.3a,b), the peel samples indicated clearer grouping compared to pulp samples, which could be caused by the lower internal variation between peel samples in the same farm compared with the variation in the pulp samples. The PCA separation was consistent with the results of the ANOVA test of the peel samples, while the above-mentioned elements and stable ratios were also significantly different among the 10 farms (Figure 3.3b). As seen from Table 3.2, the farms which showed larger differences in the scatter plot (Figure 3.2), using altitude, temperature, and rainfall, also grouped separately in the PCA plots. The relation between the separation in “geographical factors” and “PCA groups” could possibly indicate the effects of growing conditions on the stable isotopic ratios and elemental compositions of bananas. Figure 3.3 showed that organic farms DR1 and DR2 were separated from other organic farms (EC1 and PE1) in both the pulp and peel results, which could be due to the differences in geographical factors (or other external factors) such as altitude, monthly mean temperature, and annual rainfall. For example, the monthly mean temperature of DR1 and DR2 were higher than EC1 and PE1, also the rainfall of DR farms was higher than PE1 and lower than EC1.

At the same time, some values of stable isotopes and elements of organic farms were similar when compared with conventional farms. The PCA plots also showed that conventional farms and organic farms were completely separated, such as EC1 and EC2 (Figure 3.3). This phenomenon could be because organic fertilisers were also used in conventional farms for sustainable banana farming (Gonçalves & Kernaghan,

2014), which could explain why some organic farms, such as PE1, were plotted closely to conventional farm such as EC2 in the PCA results. Overall, the differences in stable isotope ratios and elemental compositions of bananas from selected countries were the result of the combined effects of the geographical factors and production systems.

3.3.5. Correlation analysis of banana compositions and their growing conditions

To protect the integrity of food and the interests of consumers, most of the related works of literature attempt to discriminate the origin of foods based on the geographical distances such as the “national or provincial boundaries”, whereas only a few papers have been published concerning the underlying mechanisms linked to geographical origin. However, both macromolecular nutrients and micro-metabolites of foods are influenced by the environmental conditions of the origin, planting methods, temperature, soil conditions, etc. (Cao et al., 2019; Sim et al., 2017). Therefore, exploring the correlations between the authentic growing conditions and the composition of foods could provide more in-depth evidence for its traceability. The banana is a suitable fruit to use for correlation research between growing conditions and compositional attributes, as most banana plantations are only located in tropical and subtropical regions, while being sold worldwide. The fresh banana is the most important fruit traded internationally. Verifying the geographical origin of bananas is important to protect the consumers’ pocket and farmers’ benefits (FAO, 2020). At the same time, identifying and tracing organic bananas (i.e., production system) could further ensure the sustainable supply of bananas and protect consumers' right to know what they are buying.

To further explain the relationships between the stable isotopes and elements of different sampling farms and the local growing conditions, the compositions and their growing conditions were correlated using Pearson's correlation coefficients (r). The relationship between growing conditions and banana compositions were shown using the network in Figure 3.4. The distance between the attributes indicated the strength of the correlation coefficient, while the blue or red colours were used to show if they were positive or negative, respectively.

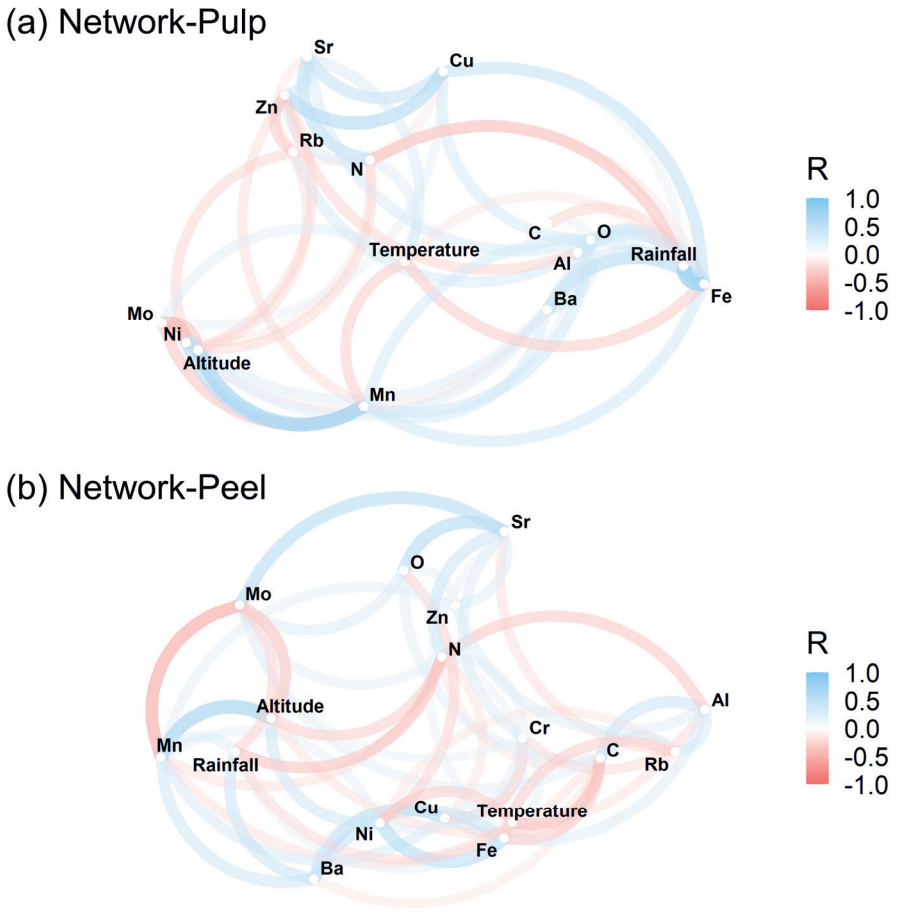


Figure 3.4. The correlation network of stable isotopes, elements, and growing conditions of banana pulp (**a**) and peel (**b**). (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

For banana pulp, a slight negative relationship was found in $\delta^{13}C$ and rainfall (r = −0.32, $p = 0.18 \times 10^{-3}$), which is in agreement with the several studies where $\delta^{13}C$ of fruits negatively correlate with the rainfall during sugar accumulation in fruit body (Camin et al., 2015). $\delta^{18}O$ of wine was reported to be highly correlated with daily temperatures and local precipitation (Martin & Martin, 2003), and the network analysis of banana pulp showed that $\delta^{18}O$ was slightly positively correlated with altitude (r = 0.33, $p = 0.14 \times 10^{-3}$) and rainfall (r = 0.33, $p = 0.12 \times 10^{-3}$) (Figure 3.4a). Tables 3.1 and 3.2 also showed that farm CR1 had the higher altitude and rainfall values than

CO1, at the same time, significantly higher $\delta^{18}O$ were detected in pulp samples from CR1. As for stable isotope N, only a weak positive correlation with temperature and $\delta^{15}N$ ($r = 0.32$, $p < 0.01$) were observed. On the other hand, the element Fe indicated a strong correlation with rainfall ($r = 0.74$, $p = 0.01$), a significant and moderate correlation was found between altitude and two elements: Mn ($r = 0.66$, $p = 0.01$) and Ni ($r = 0.53$, $p < 0.01$). A negative correlation with Fe ($r = -0.38$, $p < 0.01$) and Mn ($r = -0.36$, $p < 0.01$) due to the effects of temperatures was also found in network analysis. Generally, the differences among sampling locations were mainly caused by individual geographical features such as the altitude, temperature, and rainfall. The growth of bananas requires sufficient humidity, light, and moisture, otherwise the yield and quality of bananas would be decreased (Coltro & Karaski, 2019).

For banana peel, Figure 3.4b shows that most of the compositional attributes of peel samples were associated with geographical factors. The differences of $\delta^{13}C$ from different farms could be associated with temperature conditions ($r = -0.41$, $p = 0.90 \times 10^{-3}$), while the variation of $\delta^{15}N$ was slightly correlated with altitude ($r = -0.36$, $p = 0.42 \times 10^{-2}$) and rainfall ($r = -0.42$, $p = 0.77 \times 10^{-3}$). It could suggest that other factors likely also contribute to the variations of stable isotope N from different farms. Most of the research indicates that organic production tends to increase the values of $\delta^{15}N$ more than conventional cultivation (Nishida & Sato, 2015). The PCA plots also confirmed that organic farms could have higher values of $\delta^{15}N$. The slight negative relationship was found between rainfall and $\delta^{13}C$ ($r = -0.32$, $p = 0.18 \times 10^{-3}$). Similarly, the elements Mn ($r = 0.65$, $p < 0.01$) and Ni ($r = 0.41$, $p = 0.94 \times 10^{-3}$) showed significantly positive correlations with altitude. A slight positive relationship was found in rainfall and Ba ($r = 0.44$, $p = 0.04 \times 10^{-3}$).

The significant correlation between growing conditions of the farms and the compositional attributes (stable isotopic ratios and elemental concentrations) was an obvious indication of the relationship between growing conditions and banana compositions. The underlying reason may be that geographical factors (e.g., altitude, temperature, rainfall) affected the absorption capacity of banana roots to elements, thereby promoting or inhibiting the concentration of elements in pulp and peel. Heat stress could induce various physiological injuries to plants such as root growth inhibition, therefore the absorption of elements from soil to roots will be inhibited as well (Bita & Gerats, 2013). Subsequently, a negative correlation between temperature

and most of the elements of banana pulp and peel were observed in this research chapter. Especially for farm DR1 and DR2 with higher monthly mean temperature than most farms, the elemental contents like Ba, Cu, Fe, Mn, Ni, and Cu of pulp samples were also lower than other farms correspondingly. For the effects of altitude on fruit compositions, Crespo et al. (2010) reported that chemical composition such as vitamin C, organic acids, and anthocyanins of strawberry changed due to increasing altitude. The possible reason could be that the microclimate in a high-altitude region changed a lot in air temperature, precipitation, and evaporation compared with a low-altitude region (Bennie, Huntley, Wiltshire, Hill, & Baxter, 2008). As found in this research chapter, the altitude also correlated with Mn, Mo, Ni positively or negatively of pulp and peel samples. However, not all stable isotope and elements correlated with the growing conditions, which means more geographical factors such as soil type, sunshine data, etc., should be collected to strengthen the findings.

3.4. Conclusions

A comprehensive application of IR-MS and ICP-MS was employed to identify the isotopes and mineral elements fingerprints of Cavendish bananas from different origins. The ANOVA results demonstrated that the stable isotopic ratios and elemental concentrations changed significantly according to different farm locations. Based on the obtained PCA plots, the selected farms could be separated according to different geographical factors and production systems. This revealed that the variation among banana compositions were potentially related to their growing conditions. Moreover, the $\delta^{15}N$ values of pulp and peel were significantly higher in the organic system than the conventional system. On the other hand, relations exist between the geographical factors and the concentration of elements in bananas; particularly, Mn and Ni were significantly positively correlated with altitude. The underlying reason could be that the interaction of roots and mineral elements and the banana metabolism is affected by the growing conditions. Overall, the combination of stable isotopes and elemental compositions could be used to explore the effects of local growing conditions concerning origin. However, this feasibility study was limited to one single season and a low number of samples. It is important that more comprehensive discussions on the effects of environmental factors are strengthened in future work. In the future research, a soil map based on correlation of banana composition and growing conditions will be

generated to predict the compositional features of bananas without huge chemical database.

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Appendix B.

The following are available online at https://www.mdpi.com/article/10.3390/foods10051021/s1, Table S1: The minimum and maximum values of the stable isotope ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{18}O/^{16}O$) and elemental compositions (mg/kg) of banana pulp samples (*n* = 12). Table S2: The minimum and maximum values of the stable isotope ratios ($^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{18}O/^{16}O$) and elemental compositions (mg/kg) of banana pulp samples (*n* = 6).

CHAPTER 4

4

Exploring the effects of growing conditions on volatile and non-volatile compounds of bananas (*Musa* spp.) from different countries

This chapter is in preparation for submission:
Wang, Z., Erasmus, S. W., & van Ruth, S. M. (2021). Exploring the effects of growing conditions on volatile and non-volatile compounds of bananas (*Musa* spp.) from different countries. *In preparation for submission.*

Abstract

Currently, there is limited research on the authentication of bananas, which is not conducive to food safety and consumer interests in banana supply chains. In this study, 120 banana samples from different geographical features (e.g., altitude, monthly mean temperature, annual rainfall) and production systems (e.g., organic, conventional production) were collected. The effects of growing conditions on volatile and non-volatile compounds of bananas were investigated using volatile headspace solid-phase micro-extraction gas chromatography (HS-SPME-GC-MS) and non-volatile, direct analysis in real-time high-resolution mass spectrometry (DART-HRMS) compound analysis. A total of 18 volatile compounds and 18 non-volatile compounds were identified and subjected to principal component analysis (PCA). Based on PCA biplots, the volatile compounds could separate the geographical origins of bananas and the non-volatile compounds revealed reliable differentiation in production systems. The contributions are mainly related to the abundance of volatiles such as 3-methyl-butanol, 2-butyl-2-octenal, butanol, and 3-methyl-1-butanol-acetate and non-volatiles such as folic acid, zeaxanthin, y-tocopherol, and L-tryptophan. To explore the interaction of volatile and non-volatile compounds, correlation analysis using Kendall methods showed that contents of starch, protein, catechin, and retinol had positive or negative effects on volatile compounds. This study showed that the differences in volatile and non-volatiles compounds of bananas could be linked to effects of different growing conditions and provide indications to understand the correlation of volatile and non-volatile compounds of bananas.

Keywords

Banana, Compositions, Correlation analysis, Geographical origin, Organic production

4.1. Introduction

Typically, the banana is not a high risk target for food fraud compared to highly vulnerable products like olive oil, salmon, and dairy products (van Ruth et al., 2018). However, in recent years, a few economically driven adulteration incidents have been reported in banana supply chains. For example, non-organic bananas have been mislabelled as organic bananas to make greater profits (USDA, 2020). Besides, the integrity of banana supply chains also faces many new challenges, such as the invasion of *Fusarium wilt* disease, the conversion to organic production, and climate change (Bellamy, 2013; García-Bastidas et al., 2020; Varma & Bebber, 2019).

The food fraud vulnerability of agricultural products is influenced by their price related with the origin statement and the production system (e.g., organic or conventional farming, respectively), and the banana is no exception (van Ruth et al., 2018). The banana is already ranked as one of the most consumed fruits globally with the Cavendish banana (*Musa* spp, AAA group) known as the most widely planted commercial variety, because of its excellent taste and resistance to certain diseases (Sidhu & Zafar, 2018). Some Central and South American countries such as Colombia, Ecuador, and Costa Rica are very important banana growing and exporting countries (FAO, 2020). However, their bananas are vulnerable to food fraud as they are exported across the world primarily through complex supply chains. Therefore, the necessary authentication and traceability system of bananas is of great significance for maintaining the robustness and protecting the organic industry in the banana supply chain.

In the past few years, there have been many research papers published on food authentication and traceability systems (Wadood, Boli, Xiaowen, Hussain, & Yimin, 2020). Taking into account the progress of banana research, our previous studies have found that stable isotope ratios mass spectrometry, inductively coupled plasma mass spectrometry (ICP-MS) and hyperspectral imaging (HSI) can be used to preliminarily distinguish the banana samples from different soil conditions and climates (Chapter 2, Wang, Erasmus, Dekker, et al., 2020; Chapter 5, Wang, Erasmus, Liu, & van Ruth, 2020). However, the application of many traceability technologies is limited due to complex sample pre-treatment, expensive equipment, and time-consuming instrument debugging.

Headspace solid-phase micro-extraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) can be used for the fast identification of volatile components accompanied by the advantages of simple pre-treatment for samples. Considering the unique flavour of bananas, volatile compounds are an important indicator that distinguish bananas from other fruits. Many papers have shown that the types and concentrations of volatile components in fruits are highly related to the agroclimatic parameters of the geographical origin and are also influenced by organic agriculture. For example, the soil and climate of orchards had an effect on the concentrations of volatile compounds of Chilean virgin olive oils (Romero, Saavedra, Tapia, Sepúlveda, & Aparicio, 2016). As previously reported, the climate differences of the original area were more likely to induce metabolic variations and change the content of volatile substances, while apples from organic farms also contained more aroma components (Giannetti, Boccacci Mariani, Mannino, & Marini, 2017). Besides HS-SPME-GC-MS, direct analysis in real-time high-resolution mass spectrometry (DART-HRMS) is one of the high-resolution ambient mass spectrometry methods with rapid, high throughput, and non-targeted features. The spectra obtained from DART-HRMS could be used to construct a panoramic view of the non-volatile fraction of samples, which are helpful to find small changes from a large sample set. The DART-MS method has been used to determine poultry production history with 100% accuracy (Birse et al., 2021), evaluate the quality and authenticity of cocoa products (Rýdlová et al., 2020) and discriminate the geographical origin of beef in six countries (Wang, Xu, Wang, Chen, & Xu, 2021). Also, consumers generally prefer organic and country-of-origin foods due to reasons associated with higher nutrition, food security, and sustainable production (Santeramo & Lamonaca, 2020; Thøgersen, Pedersen, & Aschemann-Witzel, 2019). However, there are limited reports on the authentication of banana origin and the influence of the origin and production systems on banana composition.

This study was carried out to determine distinctive compositional features of the volatile and non-volatile fractions of bananas related to the geographical origin and production system using HS-SPME-GC-MS and DART-HRMS, respectively. Principal component analysis (PCA) was used to explore the patterns of volatile and non-volatile compounds. The relationships between the volatile and non-volatile compounds were

also explored. The findings aim to assist in revealing the effects of growing conditions on volatile and non-volatile profiles of bananas.

4.2. Materials and methods

4.2.1. Banana samples

In total, 120 banana samples from different countries and production systems were used in this study. To ensure the lowest influences from variety, only one type of banana variety, namely *Cavendish Williams* (*Musa* spp., AAA group), was sourced. Ten farms, coded as CO1, CR2, CR2, CR3, DR1, DR2, EC1, EC2, PA1, PE1, were selected for the study (Figure 4.1). These farms were located in different countries (Columbia, Costa Rica, Dominican Republic, Ecuador, Panama, and Peru) and practiced different production systems (organic or conventional management) (Table 4.1). Twelve banana trees were selected randomly from each farm and one banana bunch was harvested from each tree in 2018. Then, the banana from the top position in the bunch were selected, packaged, and transported to Wageningen University & Research in clean polyethene bags by courier at 11-13°C. After they arrived at the laboratory, two banana fingers from each bunch were selected to combine one sample to obtain enough samples for analysis. In total, 12 samples were selected to represent each farm. Then, the banana pulp was carefully separated from the peel and cut into small pieces. To minimize the impact of storage and transportation on banana composition, the pulp pieces were then freeze-dried and homogenized to a fine powder and stored at -20°C before analysis. On the day of analysis, the samples were defrosted at room temperature for 2 hours before the volatiles and non-volatiles analyses commenced.

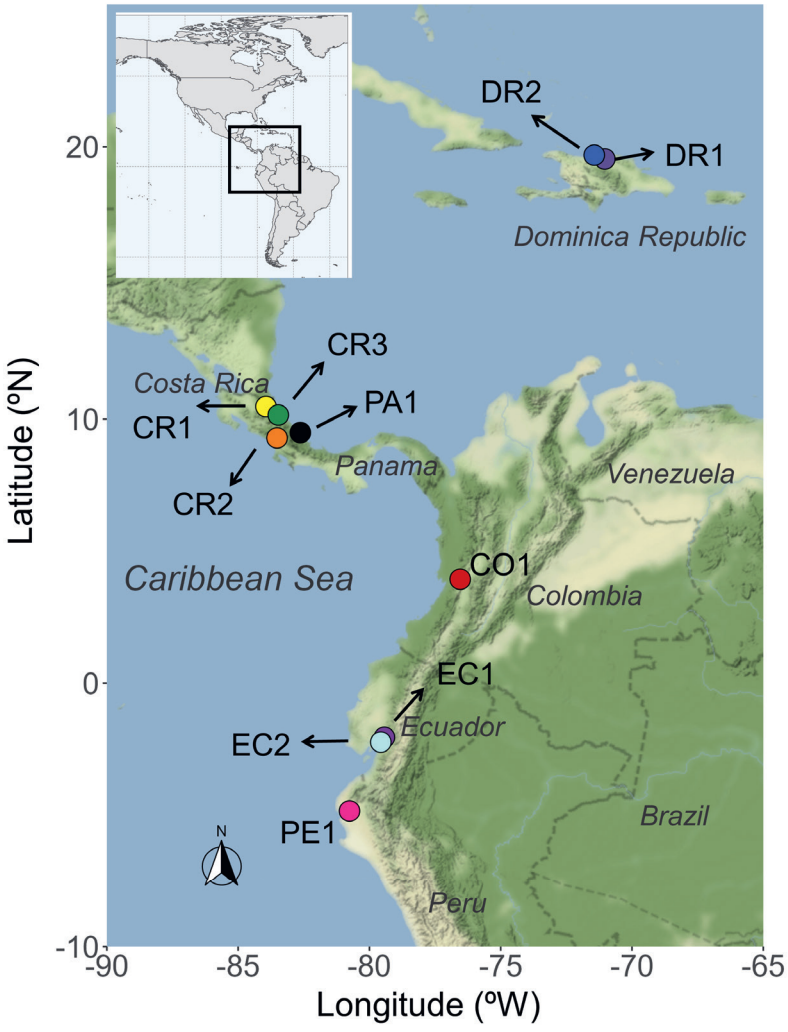


Figure 4.1. The geographical locations of the banana sampling farms. CO: Colombia; CR: Costa Rica; DR: Dominican Republic; EC: Ecuador; PA: Panama; PE: Peru; the numbers refer to the individual farms within each country. (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

4.2.2. Growing conditions

To comprehensively evaluate the effects of growing conditions on banana compositions. The altitude of each farm was obtained according to Global Positioning System (GPS) coordinates provided by the local farms. The monthly mean

temperature and annual rainfall readings were collected from the public databases CRU TS4.04 (Climatic Research Unit (CRU) Time-Series (TS) version 4.04). The details of the collected samples and related growing conditions are listed in Table 4.1.

Table 4.1. Overview of the banana samples and related growing conditions.

Country	Farm	Number of samples	Production system	Altitude (m)	Monthly mean temperature (℃)	Annual rainfall (mm/year)
Colombia	CO1	12	Conventional	66	23.2	1837
Costa Rica	CR1	12	Conventional	726	23.4	2857
	CR2	12	Conventional	47	24.4	5014
	CR3	12	Conventional	24	26.3	4378
Dominican Republic	DR1	12	Organic	65	26.7	925
	DR2	12	Organic	27	26.7	925
Ecuador	EC1	12	Organic	32	22.9	1511
	EC2	12	Conventional	22	26.5	843
Panama	PA1	12	Conventional	20	19.7	3679
Peru	PE1	12	Organic	40	24.1	200

4.2.3. Volatile compound analysis

The volatile compound profiles of banana pulp were investigated using headspace solid-phase micro-extraction gas chromatography (HS-SPME-GC-MS) as described by Ekpa et al. (2020) with some modifications. Briefly, 1 g of pulp powder with 1-octanol as internal standard (diluted in methanol to a concentration of 5 mg/L) was placed in a 10 mL glass vial and incubated at 40°C for 15 min. The volatile compounds of the banana samples were absorbed by a three-phasic (DVB-CAR-PDMS) SPME fibre (Supelco, Bellefonte, PA, USA) for 10 min. Then, the SPME fibre was desorbed in the GC injector port at 250°C for 10 min. The extracted volatile compounds were injected into the Stabilwax DA capillary column (30 m × 0.25 mm inner diameter × 0.25 μm film thickness) by helium as carrier gas at a rate of 1 mL/min. The separation program for SPME injection was 40°C for 2 min, increased at 10 °C/min to 230°C and then held at 230°C for 5 min. In the end, the separated compounds were eluted through 230°C mass-transfer lines and the mass spectrum of the individual compound was detected by the mass detector operating in an electron ionisation mode (internal ionisation source; 70 eV) with a scan range from 33 to 300 m/z (GC Clarus 500, PerkinElmer,

Norwalk, CT, USA). Each sample was measured in duplicate. The mass spectrum of individual compound was compared to the mass-spectral library of National Institute of Standards and Technology (NIST). Compounds with a similarity index > 75% were selected to ensure high conformity of the detected compounds. 1-octanol (internal standard) was used to semi-quantitatively analyse the volatile compounds by the peak area of the total ion current chromatogram (Total Ion Chromatogram, TIC). The relative content of each volatile compound identified in banana pulp was calculated by dividing the peak area of the detected volatiles by the peak area of the internal standard (1-octanol) and multiplying the result with the concentration of the 1-octanol. In the end, the relative concentration of each volatile compound was calculated by the relative content dividing the weight of banana pulp samples (Bottiroli et al., 2021), the semi-quantitative analysis was carried out according to the following formula:

$$C_b = \frac{\frac{P_b}{P_i} \times C_i}{W_b}$$

where C_b is the relative concentration (μg/mg) of the volatile compounds in bananas, P_b is the peak area of the volatile compounds in bananas, P_i is the peak area of the internal standard, C_i is the concentration of the internal standard, W_b is the weight of samples.

4.2.4. Non-volatile compound analysis

The non-volatile compounds of banana pulp were analysed by direct analysis in real-time high-resolution mass spectrometry (DART-HRMS). A Q-exactive quadrupole orbitrap high-resolution mass spectrometer (Thermo Fisher Scientific, San Jose, CA) was coupled with a DART-SVP (simplified voltage and pressure) ion source (IonSense, Saugus, MA, USA). For the setting of the DART-SVP ion source, the helium gas at a flow rate of 3.7 L/min in running mode and nitrogen was used in standby mode. Both positive and negative modes were used with a gas temperature of 350°C. The discharge needle voltage was kept at -5000 V and the grid electrode voltage was set to 350 V. The distance between the DART exit and MS inlet was at 5 mm. For the parameters of the mass spectrometer, the capillary temperature of ion source was set at 250°C. Data were acquired in full-scan type with an m/z range of 150-1000. The mass resolution was 35000 (full width at half-maximum, fwhm) and the microscan

number was set at 4. The automatic gain control (AGC) target was 3×10^{-6} and the maximum inject time was performed in 50 ms. Before samples analysis, the MS was calibrated for a mass accuracy lower than 5 ppm using the ion calibrating solution from the manufacturer. Banana pulp was sucked into the dip-it tip and was moved into DART ionization region for sample analysis. The background noise was also obtained by an empty dip-it tip. All banana pulp samples were analysed both in positive and negative mode at 30 s, respectively. The DART-HR-MS spectra of each sample were recorded in duplicate in the Xcalibur software (Thermo Fisher Scientific, San Jose, CA, USA).

4.2.5. Statistical analysis

All raw data was exported using the built-in software of the instrument for subsequent analysis. In the HS-SPME-GC-MS analysis, the chromatograms and mass spectra were processed with Xcalibur software (Thermo Fisher Scientific, San Jose, CA, USA). The identified volatile compounds were used for PCA plots. For the DART-HRMS analysis, the background was subtracted from each sample and the normalised spectrum list was extracted in Xcalibur as well. The m/z peaks were tentatively identified by matching online databases such as FooDB (https://foodb.ca/) and METLIN database (https://metlin.scripps.edu/). The PCA were also performed based on spectrum lists of identified non-volatile compounds of each sample. All the obtained raw data was pre-processed before chemometrics analysis were performed. Briefly, PCA was conducted to explore the data distribution in the different datasets using the R package *FactoMineR* and *factoextra* (Kassambara & Mundt, 2017; Lê, Josse, & Husson, 2008). In the correlation analyses, the chemical compositional data of bananas were adopted from Chapter 5 in the same sample set (Chapter 5, Wang, Erasmus, Liu, et al., 2020). The correlation coefficients between measured volatile and non-volatile compounds were conducted using the Kendall methodology (Lanzerstorfer et al., 2014). All the data analyses were performed in R 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria).

4.3. Results and discussion

4.3.1. HS-SPME-GC-MS analysis of banana pulp

In total, more than 110 volatile compounds were detected and 18 volatile compounds were tentatively identified at a similarity index above 75% in the volatile fraction of the

banana samples from different geographical origins and production systems (Table 4.2). Previous papers identified 40 compounds in fresh bananas using similar SPME methods (Bugaud & Alter, 2016). The difference that only 18 volatile compounds were identified in this research is probably because the banana samples were freeze dried before compounds extraction. The purpose of the samples being freeze-dried is to reduce the impact of the sample preservation process on the banana composition and to extend the shelf life to the needs of long-term experiments. However, to the best of our knowledge, the main volatile compounds can still remain in the banana powder after different drying methods such as vacuum belt drying, freeze-drying and air-drying. Especially characteristic aroma components of bananas such as 3-methylbutyl acetate and butanoic acid 3-methylbutyl ester were also identified in the freeze-dried pulp powder (Wang, Li, Chen, Bao, & Yang, 2007). The results in Table 4.2 showed that four types of volatiles were identified, specifically alcohols, aldehydes, organic acids, and esters. In the volatile compounds of bananas, esters have been reported as the major contribution of banana volatiles in some previous studies (Facundo, Garruti, Dias, Cordenunsi, & Lajolo, 2012; Jha, Zhang, Hayashi, & Liu, 2022). The ester fractions such as 3-methyl-1-butanol-acetate were also found in this study, which is consistent with findings of other published papers about the volatile compounds of bananas (Aurore, Ginies, Ganou-parfait, Renard, & Fahrasmane, 2011; Facundo, Garruti, Dias, Cordenunsi, & Lajolo, 2012; Wang, Li, Chen, Bao, & Yang, 2007; Zhu et al., 2018). Only two alcohols, butanol and 3-methyl-butanol, were detected in the banana pulp samples, which are also important volatile compounds contributing fruity notes (Table 4.2). Two aldehydes including hexanal and 2-butyl-2-octenal and six organic acids such as acetic acid, butanoic acid, pentanoic acid, hexanoic acid, heptanoic acid, and octanoic acid were also identified. As previously reported, the presence of aldehydes and organic acids likely account for the fruity odour of bananas (Dong, Chen, Wang, Huang, & Yi, 2014).

Table 4.2. The volatile organic compounds tentatively identified in the banana pulp samples by headspace solid-phase micro-extraction gas chromatography mass spectrometry (HS-SPME-GC-MS).

No.	Compound	RT (min)
	Alcohols	
1	Butanol	3.82
2	3-Methyl-butanol	4.38
	Aldehydes	
3	Hexanal	7.12
4	2-Butyl-2-octenal	15.35
	Organic acids	
5	Acetic acid	12.44
6	Butanoic acid	14.66
7	Pentanoic acid	15.94
8	Hexanoic acid	17.13
9	Heptanoic acid	18.29
10	Octanoic acid	19.69
	Esters	
11	Ethyl acetate	3.93
12	Methyl propionate	4.17
13	Methyl isovalerate	5.89
14	3-Methyl-1-butanol-acetate	7.65
15	Methyl hexanoate	8.83
16	Hexyl acetate	10.02
17	Methyl nonanoate	13.21
18	Methyl decanoate	14.38

(No.) Number; (RT) retention time.

Table 4.3. Concentration of volatile compounds identified in banana pulp with the internal standard 1-octanol (10^{-5} µg/kg).

Farm	Butanol	3-Methyl-butanol	Hexanal	2-Butyl-2-octenal	Acetic acid	Butanoic acid	Pentanoic acid	Hexanoic acid	Heptanoic acid	Octanoic acid
CO1	$43^{b} \pm 16$	$30_{c} \pm 8$	$5385^{b} \pm 216$	$111^{c} \pm 42$	$334^{d} \pm 18$	$306^{cd} \pm 17$	$81^{e} \pm 7$	$840^{d} \pm 79$	$41^{d} \pm 10$	$374^{b} \pm 47$
CR1	$29^{b} \pm 13$	$31_{c} \pm 6$	$3312^{b} \pm 127$	$90^{c} \pm 34$	$155^{d} \pm 51$	$788^{c} \pm 89$	$7.9^{e} \pm 0$	$6784^{bc} \pm 947$	$17^{d} \pm 3$	$269^{b} \pm 10$
CR2	$19^{c} \pm 3$	$60_{b} \pm 12$	$3056^{b} \pm 882$	$79^{c} \pm 35$	$173^{d} \pm 22$	$118^{d} \pm 28$	$19^{e} \pm 1$	$10259^{b} \pm 754$	$33^{d} \pm 6$	$277^{b} \pm 15$
CR3	$14^{c} \pm 3$	$96_{b} \pm 7$	$4158^{b} \pm 279$	$120^{c} \pm 20$	$174^{d} \pm 19$	$182^{d} \pm 63$	$33^{de} \pm 10$	$16000^{a} \pm 3410$	$80^{d} \pm 11$	$701^{b} \pm 55$
DR1	$100^{bc} \pm 29$	$164_{bc} \pm 10$	$5979^{b} \pm 433$	$80^{c} \pm 4$	$2064^{c} \pm 102$	$1645^{b} \pm 582$	$5665^{a} \pm 309$	$2818^{cd} \pm 974$	$831^{b} \pm 61$	$1094^{b} \pm 786$
DR2	$13^{c} \pm 5$	$580_{a} \pm 46$	$5082^{b} \pm 475$	$443^{c} \pm 7$	$6816^{c} \pm 359$	$5137^{a} \pm 121$	$4396^{b} \pm 154$	$619^{d} \pm 84$	$137^{cd} \pm 15$	$2770^{ab} \pm 284$
EC1	$210^{b} \pm 13$	$326_{b} \pm 35$	$60821^{a} \pm 5670$	$3275^{a} \pm 337$	$26468^{b} \pm 1019$	$2043^{b} \pm 66$	$89^{e} \pm 22$	$286^{d} \pm 75$	$270^{cd} \pm 74$	$4580^{a} \pm 562$
EC2	$518^{a} \pm 31$	$541_{a} \pm 41$	$64401^{a} \pm 5362$	$2156^{b} \pm 110$	$87813^{a} \pm 3923$	$444^{cd} \pm 19$	$199^{de} \pm 34$	$1051^{d} \pm 104$	$550^{bc} \pm 388$	$4802^{a} \pm 432$
PA1	$47^{bc} \pm 5$	$114_{bc} \pm 12$	$8382^{b} \pm 1036$	$148^{c} \pm 12$	$3560^{d} \pm 944$	$217^{d} \pm 51$	$696^{d} \pm 12$	$531^{d} \pm 164$	$1925^{a} \pm 136$	$849^{b} \pm 103$
PE1	$34^{bc} \pm 4$	$59_{c} \pm 6$	$5090^{b} \pm 899$	$46^{c} \pm 6$	$50^{d} \pm 8$	$1930^{b} \pm 59$	$3680^{c} \pm 533$	$379^{d} \pm 115$	$72^{d} \pm 4$	$748^{b} \pm 92$

Table 4.3. (continued)

Farm	Ethyl acetate	Methyl propionate	Methyl isovalerate	3-Methyl-1-butanol-acetate	Methyl hexanoate	Hexyl acetate	Methyl nonanoate	Methyl decanoate
CO1	33240^{a} ± 1607	14^{c} ± 3	78^{d} ± 4	1552^{f} ± 149	11240^{a} ± 4043	751^{b} ± 58	191^{b} ± 45	3525^{b} ± 702
CR1	110^{c} ± 10	26^{c} ± 9	308^{c} ± 13	397^{g} ± 15	7240b^{c} ± 106	1166^{b} ± 97	115^{b} ± 19	2427^{c} ± 374
CR2	151^{c}± 81	29^{c} ± 9	205^{cd} ± 53	299^{g} ± 11	6025^{c} ± 928	308^{b} ± 14	128^{b} ± 19	6555^{a}±316
CR3	21330^{b} ± 4729	20^{c} ± 8	777^{a} ± 30	420^{g} ± 11	5846^{c} ± 1761	13438^{a} ± 4042	133^{b} ± 29	1688^{c}±133
DR1	302^{c} ± 23	51^{c} ± 11	636^{ab} ± 63	4439^{cd} ± 817	3695^{d} ± 775	3845^{b} ± 140	679^{b} ± 27	5549^{a}±102
DR2	244^{c} ± 43	525^{c} ± 112	579^{ab} ± 48	5275^{a} ± 757	2662^{d} ± 215	656^{b} ± 35	832^{a} ± 96	4220^{b}±605
EC1	1668^{c} ± 94	25^{c} ± 3	314^{c} ± 11	3574^{e} ± 477	8356^{b} ± 235	553^{b} ± 207	342^{b} ± 21	4062^{b}±541
EC2	52^{c} ± 3	756^{c} ± 96	518^{b} ± 19	3783de ± 484	11800^{a} ± 3561	596^{b} ± 36	407^{b} ± 80	4300^{b}±549
PA1	306^{c} ± 21	10764^{b} ± 3742	283^{c} ± 27	4680^{bc} ± 163	3522^{d} ± 750	9703^{a} ± 12778	833^{a} ± 28	1950^{c}±340
PE1	26^{c} ± 9	17718^{a} ± 5027	296^{c} ± 74	3541^{e} ± 100	3033^{d} ± 449	2290^{b} ± 4041	63^{b} ± 4	1740^{c}±306

The semi-quantitative concentration of each compound was presented as the relative quantity compared to the internal standard 1-octanol (10^{-5} μg/kg) (Table 4.3). The relatively high variance may be caused by the limited number of samples from each farm. For the differences in geographical origins, the contents of volatile compounds were all influenced by different geographical features such as altitude, rainfall, and temperature. For example, the content of volatile compounds in CR1, CR2, and CR2 were close, which may be the result of similar geographical features in altitude, rainfall, and temperature among these farms. However, some volatile contents such as ethyl acetate, methyl propionate, methyl isovalerate, and 3-methyl-1-butanol-acetate were significantly different between the farms CO1, CR1, and PA1. Correspondingly, the geographical features of these farms are also different. For example, the altitude of CR1 is much higher than PA1 and rainfall of CR1 is lower than most of the conventional farms. Therefore, it can be inferred that altitude, temperature, and rainfall can affect the volatile content of bananas from different farms. As reported, the growing features may affect the growth process of fruits, resulting in compositional differences (Spinardi, Cola, Gardana, & Mignani, 2019). The previous research on bananas indicated that banana compositions such as moisture, starch, fibre were associated with local growing conditions such as altitude, temperature, and rainfall (Chapter 2, Wang, Erasmus, Dekker, et al., 2020; Chapter 5, Wang, Erasmus, Liu, et al., 2020). Bozoudi et al. (2019) also reported that the volatile compounds of mountainous plant species could be influenced by farm locations. The findings in this research paper are also consistent with reported results in volatile compounds in apple juices (Medina, Perestrelo, Santos, Pereira, & Câmara, 2019) and olive oil (Youssef et al., 2011), which suggest that geographical features could influence volatile formation and specific volatile compounds could also be used as potential indicators of geographical origin of food products.

For the differences of production systems, as seen in Table 4.3, the volatile compounds from organic farms such as EC1, DR1, DR2 were significantly higher than most of the other farms, especially conventional farms CO1, CR1, CR2, and CR3. For example, the alcohols, aldehydes, and carboxylic acids were the main volatile groups affected by the production system, presenting higher levels in the banana pulp cultivated in organic farms than in conventional farms. Regarding organic cultivation is used in above farms, the differences in volatiles showed that the organic production

contributes to the accumulation of some volatile substances. There are also studies showing that the organic and conventional farming can lead to difference in quality and nutritional value of fruits (Maggio, De Pascale, Paradiso, & Barbieri, 2013; Pacheco et al., 2017; Santarelli et al., 2020). However, the effects of conventional production (e.g., CR1, CR2, CR3) could increase the contents of esters in banana pulp compared to organic production (e.g., PE1, DR1, DR2). The findings in this research are in agreement with studies on the effects of organic production on the volatiles of orange juices (Cuevas, Pereira-Caro, Moreno-Rojas, Muñoz-Redondo, & Ruiz-Moreno, 2017), and similarly for apples (Giannetti, Boccacci Mariani, Mannino, & Marini, 2017). Overall, the impact of the production system on the differences of volatile compounds were highly dependent on the types of volatiles (Cuevas, Moreno-Rojas, Arroyo, Daza, & Ruiz-Moreno, 2016).

A PCA was conducted on the identified compounds to visualise the differences in grouping of bananas from different farms. PC1 and PC2 explained 32.1% and 15.8% of the total variation, respectively. Figure 4.2 showed that the combination of PC1 and PC2 could tentatively separate the banana groups according to their geographical origins and production systems - with the organic farms located largely on the left side along PC1. Loading plots showed that the main contributions were from 3-methyl-butanol, 2-butyl-2-octenal, butanol, 3-methyl-1-butanol-acetate, pentanoic acid, methyl decanoate, and hexanoic acid. Regarding all the banana samples were harvested at the same ripening stage and shipped to the laboratory at low temperature, the impact of seasons and post-harvest storages on banana compositions has been minimized. Similar research on volatile compounds of apple juices using PCA plots also confirmed the effects of growing conditions (Medina, Perestrelo, Santos, Pereira, & Câmara, 2019). Viewed from the country's resolution, the farms from Dominican Republic (DR1, DR2) and Ecuador (EC1, EC2) grouped separately from the samples of the other countries. The likely reason could be that the volatile organic compounds were influenced by the local climate features. Furthermore, the geographical distance could have resulted in complex variations when one considers the information on the growing conditions from Table 4.1. The greater difference in growing conditions could introduce the greater distance of different samples on the PCA plots. Correspondingly, viewed from the farm's resolution, the farm CO1 and CR3 were separated well from each other, and Table 4.1 also showed that they had obvious differences in altitude and rainfall.

However, some farms such as EC1, EC2, PA1, PE1 were still overlapping more than above examples, which is possibly due to the similar parameters in altitude and rainfall. For the PCA results in production systems, most of samples could be separated well according to conventional and organic systems despite some overlap (Figure 4.2b). The main contribution was from the different contents of volatiles such as 3-methyl-butanol, 2-butyl-2-octenal, butanol, and 3-methyl-1-butanol-acetate. For example, the organic farms DR1, DR2 were located in the left side along the PC1 and the conventional farms CO1, CR1, CR2, CR3 in the right side. The production system differences were also in agreement with the results in Table 4.3. The current finding in this research paper suggested that the differences in volatile compounds could indicate the production systems of banana farms, which is in agreement with published research on volatile compounds of passion fruit (Macoris, Janzantti, Garruti, & Monteiro, 2011) and basil (Klimánková et al., 2008).

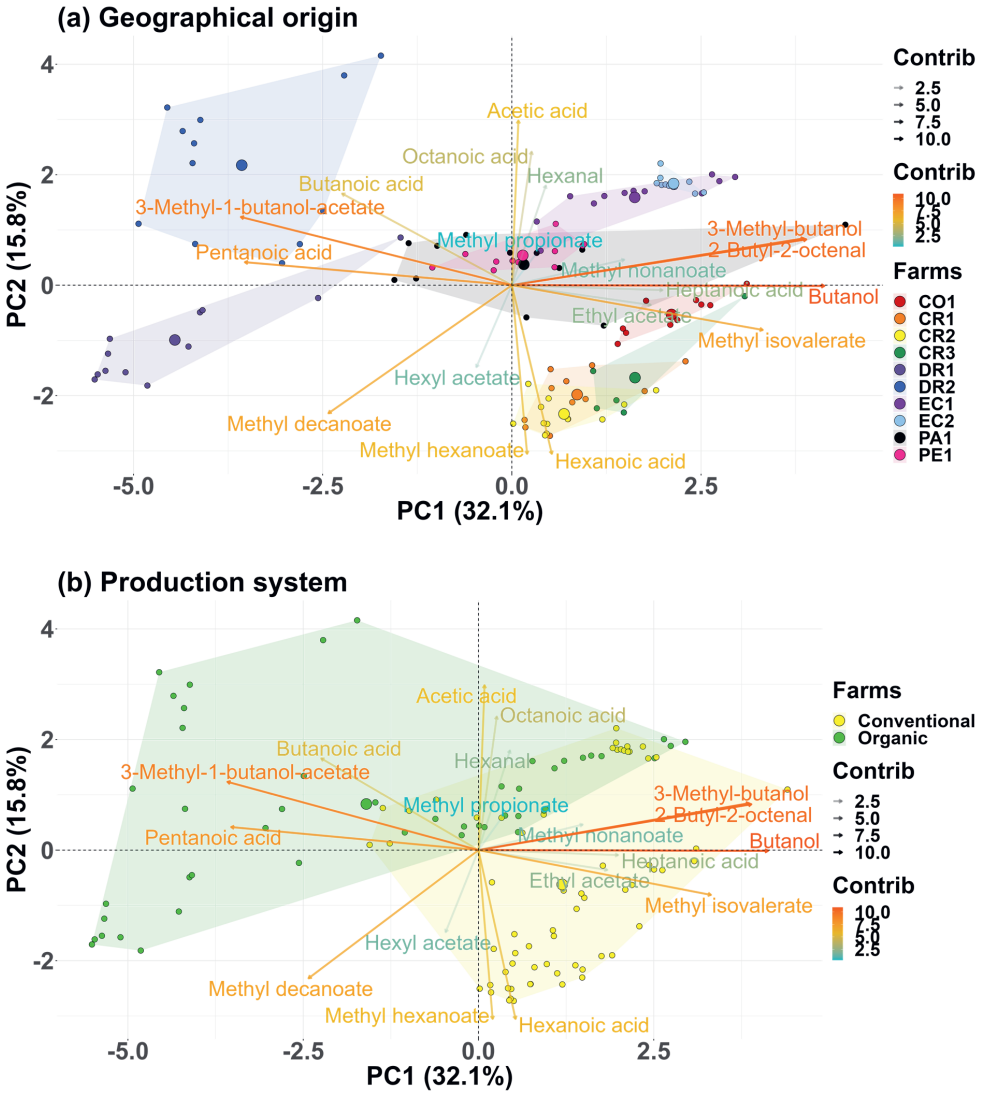


Figure 4.2. Principal component analysis (PCA) biplots based on the volatile compounds of banana pulp samples from different geographical origins (a) and production systems. (b). CO: Colombia; CR: Costa Rica; DR: Dominican Republic; EC: Ecuador; PA: Panama; PE: Peru; n = 12 samples per farm. (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

4.3.2. DART-HRMS analysis of banana pulp

The representative DART-HRMS spectra obtained from the positive and negative modes are shown in Figure 4.3. The identified compounds according to their mass intensity are listed in Table 4.4. The banana pulp from different geographical origins and production systems shared similar mass intensity for most of the non-volatile compounds. However, there were differences in the mass intensity among samples from different farms. It is reported that the mass intensity from DART-HRMS could be used to comprise integrity information of food products such as the retrospective control of feed fraud (Cajka, Danhelova, Zachariasova, Riddellova, & Hajslova, 2013), geographical origin determination of *Angelica gigas* roots (Kim et al., 2015), and the authentication of organic tomatoes (Novotná et al., 2012). Therefore, the mass intensity differences in banana samples may provide relevant information on the effects of different growing conditions. For example, the bananas from CO1, CR1, CR2, PA1 had significantly higher sucrose contents compared with PE1, DR1, DR2, and also a higher annual rainfall. This indicates that rainfall may influence the non-volatile contents of bananas. Regarding the effect of monthly mean temperature, the farm PA1 had the lowest temperature and DR1, DR2 had the highest temperatures. However, most of the non-volatile compounds showed significant differences in mass intensity for these three farms. A similar effect was also found for altitude, where high-altitude farms, such as CR1, showed significant differences in mass intensity regarding the α-carotene, α-tocopherol, α-tocotrienol, catechin, and d-tocopherol contents when compared with other low-altitude farms. The difference of production systems was also shown between farm EC1 and EC2, for example, EC1 practising an organic system had significantly higher intensity in kaempferol and retinol than EC2 which used a conventional production system. Furthermore, it is reported that the effects of growing conditions could be a combined effect from geographical features and production system (Pacheco et al., 2017). Therefore, the normalised TIC spectrum list of each sample in both positive and negative ionization mode was exported for PCA plotting. The preliminary PCA was conducted to show the data distribution of each banana sample according to influences from geographical origins and production systems (Figure 4.4).

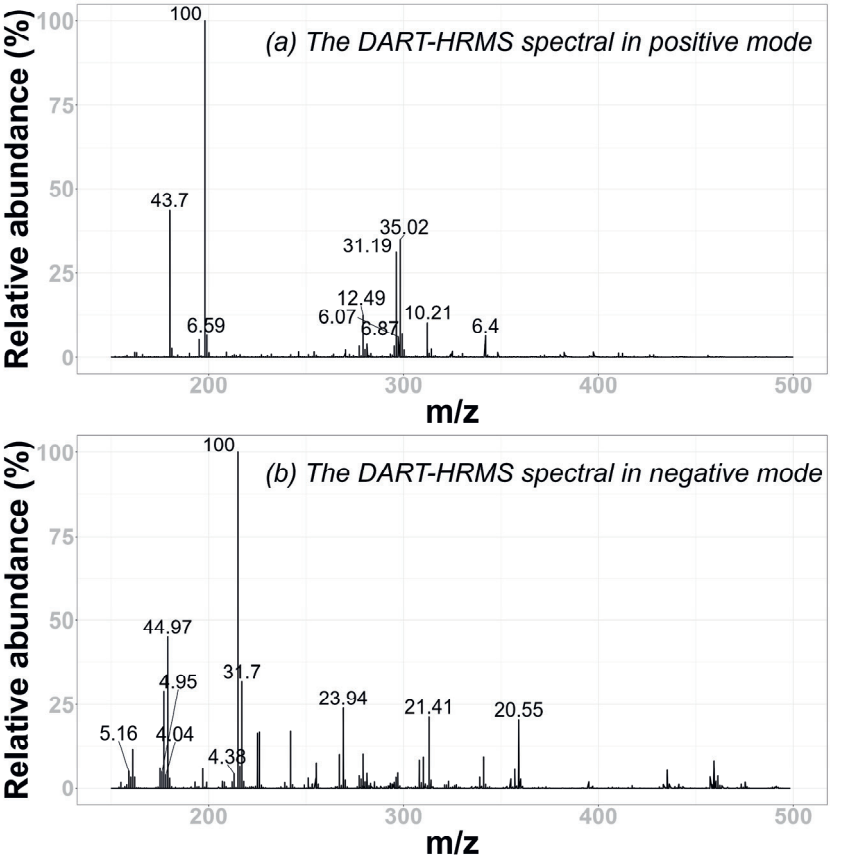
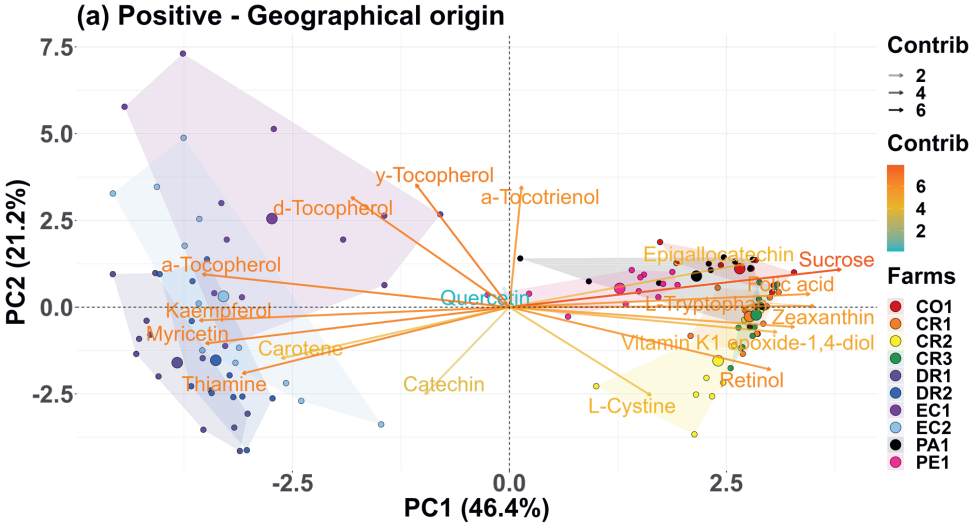


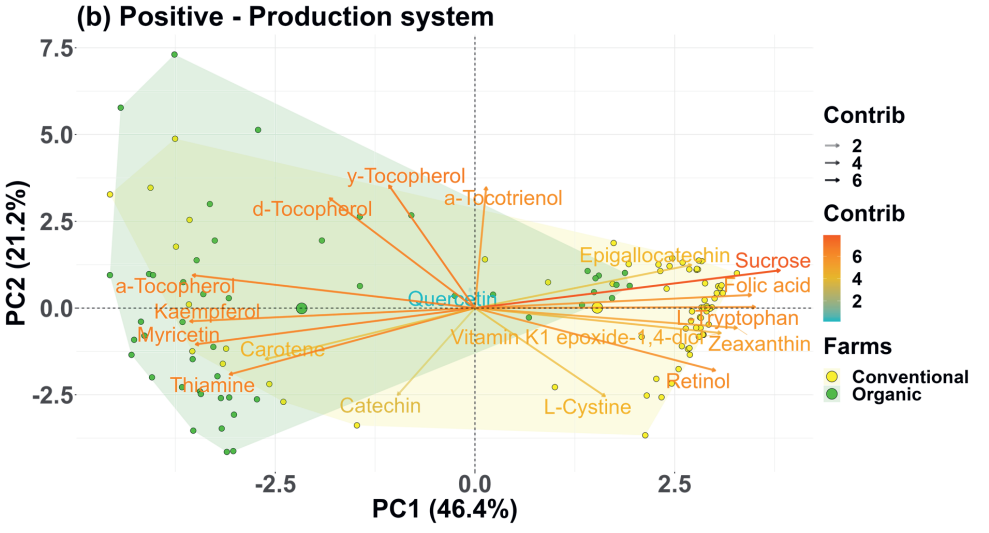
Figure 4.3. Representative direct analysis in real-time high-resolution mass spectrometry (DART-HRMS) spectra acquired in positive (a) and negative (b) ionization modes for the banana pulp samples.

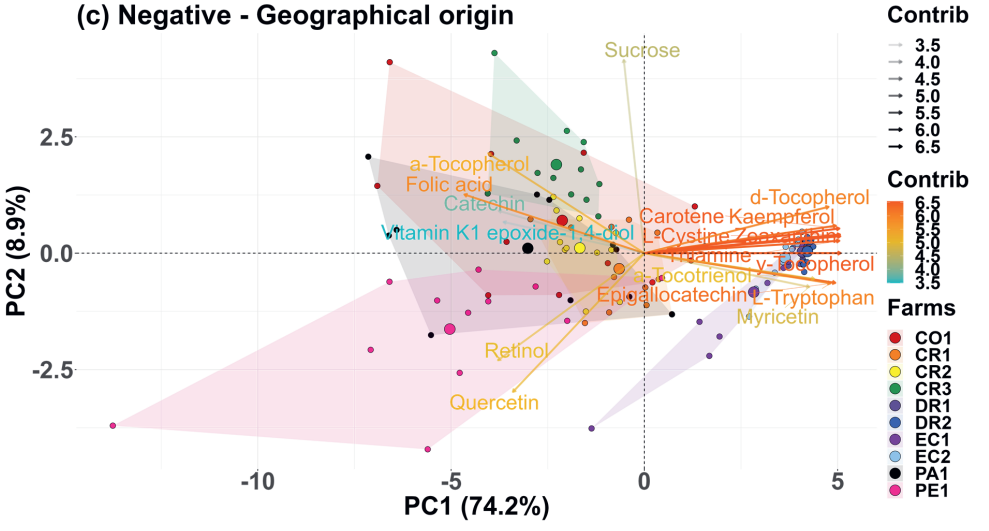
Table 4.4. The tentatively identified non-volatile compounds and their mass intensity in bananas among different farms.

Name	m/z	CO1	CR1	CR2	CR3	DR1	DR2	EC1	EC2	PA1	PE1
Carotene	536.4	6040^{d} ± 361	4047^{d} ± 404	3050^{d} ± 305	2054^{d} ± 205	91184^{a} ± 118	321^{d} ± 32	19809^{c} ± 725	83180^{a} ± 1025	51494^{b} ± 1025	45753^{b} ± 1587
Tocopherol	430.3	43031^{e} ± 1258	40069^{e} ± 1258	38588^{e} ± 4528	37107^{e} ± 8798	245169^{a} ± 12589	141178^{c} ± 47853	173988^{b} ± 12589	40405^{e} ± 1258	107197^{d} ± 75236	193173^{b} ± 45823
Tocotrienol	424.3	15321^{a} ± 3258	7829^{c} ± 125	4084^{ef} ± 98	338^{f} ± 253	2620^{f} ± 225	1185^{f} ± 99	10437^{b} ± 1587	762^{f} ± 55	5599de ± 1472	1902^{ef} ± 526
Catechin	290.0	66^{d} ± 5	213538^{c} ± 125896	320273^{b} ± 78536	427009^{a} ± 8854	2404^{d} ± 123	1^{d} ± 0	26^{d} ± 1	33^{d} ± 0	13^{d} ± 0	1203^{d} ± 205
Tocopherol	402.3	61088^{c} ± 9925	32794^{d} ± 1596	18648^{df} ± 7452	4501^{f} ± 305	97459^{b} ± 458	30229^{d} ± 853	165711^{a} ± 24886	27314^{d} ± 1247	96512^{b} ± 9568	63844^{c} ± 1287
›igallocatechin	306.0	203407^{a} ± 10258	104477^{b} ± 1257	55012^{c} ± 489	5547^{d} ± 387	3318^{d} ± 14	56^{d} ± 0	951^{d} ± 25	56^{d} ± 11	504^{d} ± 22	1687^{d} ± 333
Folic acid	441.1	1770^{c} ± 250	1930^{c} ± 258	2010^{c} ± 246	2090^{c} ± 77	393^{f} ± 49	222^{f} ± 12	4358^{a} ± 78	871^{d} ± 25	2614^{b} ± 22	308^{f} ± 11
Tocopherol	416.3	45998^{a} ± 423	23044^{a} ± 4398	11567^{c} ± 1253	90^{f} ± 20	2419^{df} ± 450	2443^{d} ± 349	368^{f} ± 25	8932^{cd} ± 336	4650^{df} ± 452	2431^{df} ± 88
Kaempferol	286.0	7092^{b} ± 897	8014^{b} ± 159	8475^{b} ± 523	8936^{b} ± 787	160^{c} ± 59	125^{c} ± 25	19331^{a} ±8859	0^{c} ± 0	9666^{b} ± 669	80^{c} ± 12
L-Cystine	240.0	43^{c} ± 12	29^{d} ± 12	22de ± 1	15^{e} ± 3	95^{a} ± 15	24de ± ± 9	30^{d} ± 12	3^{f} ± 0	17^{e} ± 2	59^{b} ± 8
Tryptophan	204.0	88431^{d} ± 12548	297611^{c} ± 56897	402201^{b} ± 785	506791^{a} ± 12574	33^{e} ± 25	105^{e} ± 56	2827^{e} ± 123	14^{e} ± 0	1420^{e} ± 100	69^{e} ± 25
Myricetin	318.2	759^{e} ± 198	8975de ± 258	13082^{d} ± 4448	17190^{d} ± 521	3320^{e} ± 120	31585^{c} ± 587	121774^{a} ± 3869	33818^{c} ± 1457	77796^{b} ± 7963	17453^{d} ± 8547
Quercetin	302.0	30^{e} ± 9	47^{cd} ± 5	55^{bc} ± 22	63^{b} ± 23	109^{a} ± 10	30^{f} ± 8	51^{bc} ± 1	37de ± 2	44^{cde} ± 13	54^{bc} ± 21
Retinol	286.4	10936^{c} ± 9875	6528^{cd} ± 875	4324^{cd} ± 587	2120^{cd} ± 119	113^{d} ± 30	105^{d} ± 20	102200^{a} ± 74256	87254^{b} ± 6656	94727^{ab} ± 5267	56^{d} ± 16
Sucrose	342.1	1423581^{a} ± 320589	711791^{b} ± 25897	355896^{c} ± 45894	1^{d} ± 0	153^{d} ± 52	45^{d} ± 14	50643^{a} ± 1354	39^{d} ± 10	25341^{a} ± 7358	99^{d} ± 11
Thiamine	265.1	34152^{a} ± 2587	495290^{b} ± 77585	725859^{c} ± 75236	956428^{b} ± 78625	105^{e} ± 35	3709^{d} ± 753	8552^{d} ± 250	8708^{d} ± 563	8630^{d} ± 306	1907^{d} ± 85
Vitamin K1 poxide-1,4-diol	450.3	35^{e} ± 14	609^{d} ± 25	896^{d} ± 69	1183^{c} ± 33	7283^{a} ± 452	894^{d} ± 123	1397^{c} ± 158	63^{e} ± 10	730^{d} ± 86	4088^{b} ± 652
Zeaxanthin	568.8	87^{c} ± 10	514^{b} ± 102	727^{b} ± 98	941^{b} ± 77	6430^{a} ± 252	409^{b} ± 100	505^{b} ± 102	94^{c} ± 18	300^{ab} ± 152	3419^{a} ± 752

The PCA biplot was used to show the data distribution of the samples according to growing conditions and contribution of non-volatile compounds in positive and negative modes, respectively. In the positive-mode results, the combination of PC1 and PC2 explained 67.6% of the total variance for samples from different countries. A moderate visual clustering of the samples from different farms based on their geographical origin was observed in the positive mode. For example, the farm EC2 had the tendency to separate from other conventional farms such as CO1 and CR1. While, overlapping was also be found in EC1 and EC2, and DR1 and DR2 for geographical origins, which could be caused by closely geographical features because these farms were from the same country (Ecuador and Dominican Republic, respectively). The loading plot indicated that the most contribution for geographical origin were from sucrose, γ-tocopherol, d-tocopherol, α-tocopherol, thiamine, and carotene. However, relatively big overlapping regions were observed in the sample distributions according to their production systems (Figure 4.4b). The results may be due to the similarities of geographical features offsetting the differences in different production systems. After all, EC2 farms have very similar altitude and temperature recordings compared with other organic farms such as EC1, DR1, and DR2.




(b) Positive - Production system
PC2 (21.2%)
PC1 (46.4%)
7.5
5.0
2.5
0.0
-2.5
-2.5
0.0
2.5
y-Tocopherol
d-Tocopherol
a-Tocotrienol
Epigallocatechin
Sucrose
Folic acid
a-Tocopherol
Quercetin
Kaempferol
Myricetin
Carotene
Vitamin K1 epoxide-1,4-diol
Zeaxanthin
Thiamine
Retinol
Catechin
L-Cystine
Contrib
2
4
6
Contrib
6
4
2
Farms
Conventional
Organic


(c) Negative - Geographical origin
PC2 (8.9%)
PC1 (74.2%)
2.5
0.0
-2.5
-10
-5
0
5
Sucrose
a-Tocopherol
Folic acid
Catechin
Vitamin K1 epoxide-1,4-diol
d-Tocopherol
Carotene
Kaempferol
L-Cystine
Zeaxanthin
Thiamine
y-Tocopherol
a-Tocotrienol
Epigallocatechin
L-Tryptophan
Myricetin
Retinol
Quercetin
Contrib
3.5
4.0
4.5
5.0
5.5
6.0
6.5
Contrib
6.5
6.0
5.5
5.0
4.5
4.0
3.5
Farms
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1

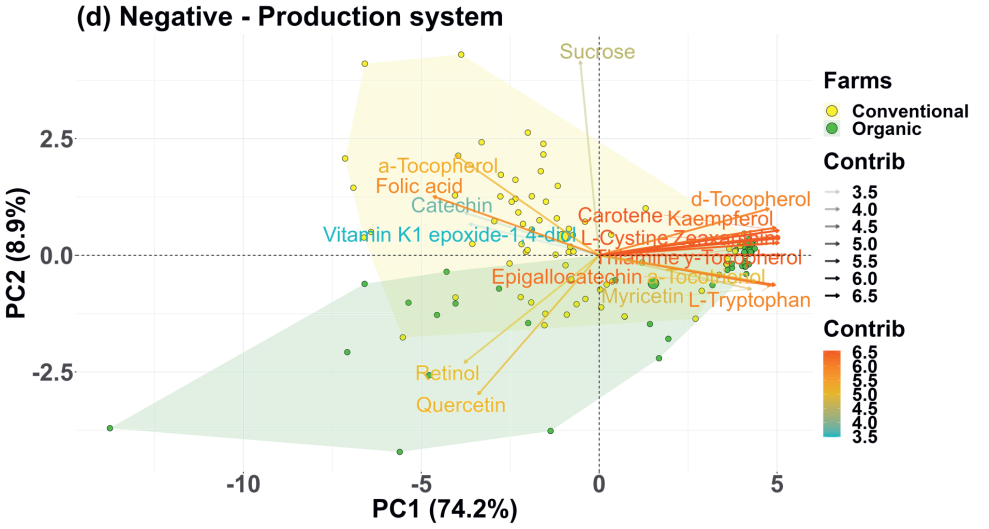


Figure 4.4. Principal component analysis (PCA) biplots of banana samples from different geographical origins and production systems based on the direct analysis in real-time high-resolution mass spectrometry (DART-HRMS) results. (a) the PCA biplot for positive mode from different farms; (b) the PCA biplot for positive mode from organic and conventional system; (c) the PCA biplot for negative mode from different farms; (d) the PCA biplot for negative mode from organic and conventional system. CO: Colombia; CR: Costa Rica; DR: Dominican Republic; EC: Ecuador; PA: Panama; PE: Peru. (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

There are similar sample distributions in the PCA biplot based on the results in negative mode. Although PC1 and PC2 explained 83.1% of the total variance, more overlapping was found on the basis of geographical origins. However, a more discrete visual clustering of production systems could be observed in the negative mode (Figure 4.4d). The main driver for separating the organic and conventional farms were differences in folic acid, zeaxanthin, y-tocopherol, and L-tryptophan according to the loadings plot. The finding in this paper is consistent with research about geographical origin discrimination of *monofloral* honeys, where the overlapping regions in PCA plots often predict close compositional features in the sample (Lippolis et al., 2020). The univariate analysis in this paper also indicated that some farms have close signal intensities for the characteristic non-volatile components (Table 4.4). The limitation of

PCA indicated that the exploratory analysis only based on DART-HRMS were not sensitive enough for clustering the effects of growing conditions on bananas, hence, discriminate model development is of interest for further investigation to achieve better discrimination between the banana features and related growing conditions. However, this will also require extension of the sample set.

4.3.3 Correlation analysis between volatile and non-volatile compounds

In the previous sections, the geographical origins and production systems of bananas were explored according to their compositional characteristics in volatile and non-volatile compounds. The ANOVA results and PCA plots indicated that the growing conditions such as altitude, rainfall, temperature, organic, conventional systems of farms could influence the contents of volatile and non-volatile compounds in banana samples. The finding in this paper agrees with most of the published literature which focuses on verifying authenticity and provenance to protect high-value agricultural products based on a large amount of analytical data such as chemical composition and volatile compounds (Drivelos, Danezis, Halagarda, Popek, & Georgiou, 2021). However, there are very few papers to report the relationship between volatile and non-volatile compounds to further explore the potential mechanism underlying the compositional characteristics of bananas. Therefore, the correlation analysis between the volatile and non-volatile compounds was conducted.

In total, 40 compositional features of the bananas were included in the correlation analysis, and the significance (p) of correlations between the volatile and non-volatile compounds were calculated. As well known, the volatile compounds of bananas were produced from the metabolism of banana pulp during the maturation process. The non-volatile compounds such as protein, lipid-like molecules, and amino acids might play a critical role in the formation of volatile compounds (Xu et al., 2021). The correlated heatmap between volatile and non-volatile compounds is presented in Figure 4.5. Starch correlated significantly with most of the volatile compounds ($r > 0.5$, $p < 0.05$). This may be due to the fact that starch is the main component of banana pulp. Many other chemicals originate from the hydrolysis and transformation of starch. However, total dietary fibre was significantly negatively correlated with most of volatile compounds such as methyl decanoate ($r = 0.63$, $p < 0.05$), ethyl acetate acid ($r = 0.45$, $p < 0.05$), which suggest that the presence of dietary fibre has a limited effect on the

generation of volatile components. In general, the characteristic volatile compound, 3-methyl-1-butanol-aceatate, had a strong and significant negative correlation with catechin ($r = -0.79$, $p < 0.05$) and d-tocopherol ($r = -0.87$, $p < 0.05$), implying that the typical banana flavour could be inhibited by some non-volatile compounds. Moreover, the non-volatile compounds such as folic acid had slightly positive correlation with most of the volatile compounds but zeaxanthin and L-cystine were found to correlate negatively with most of the volatile compounds. This indicates that the formation of banana volatiles could be comminated results of many different metabolites and their influencing factors coming from the geographical origins and production systems. Comparable results was reported in a study on apple fruit, where the contents of volatile compounds of apple were influenced by the geographical origin (e.g., farm location) and production systems (e.g., organic and conventional production systems) (Giannetti, Boccacci Mariani, Mannino, & Marini, 2017). The findings in the correlation analyses also highlight the potential use of mass spectral fingerprints for further explore the banana compositional characteristics and following effects of growing conditions.

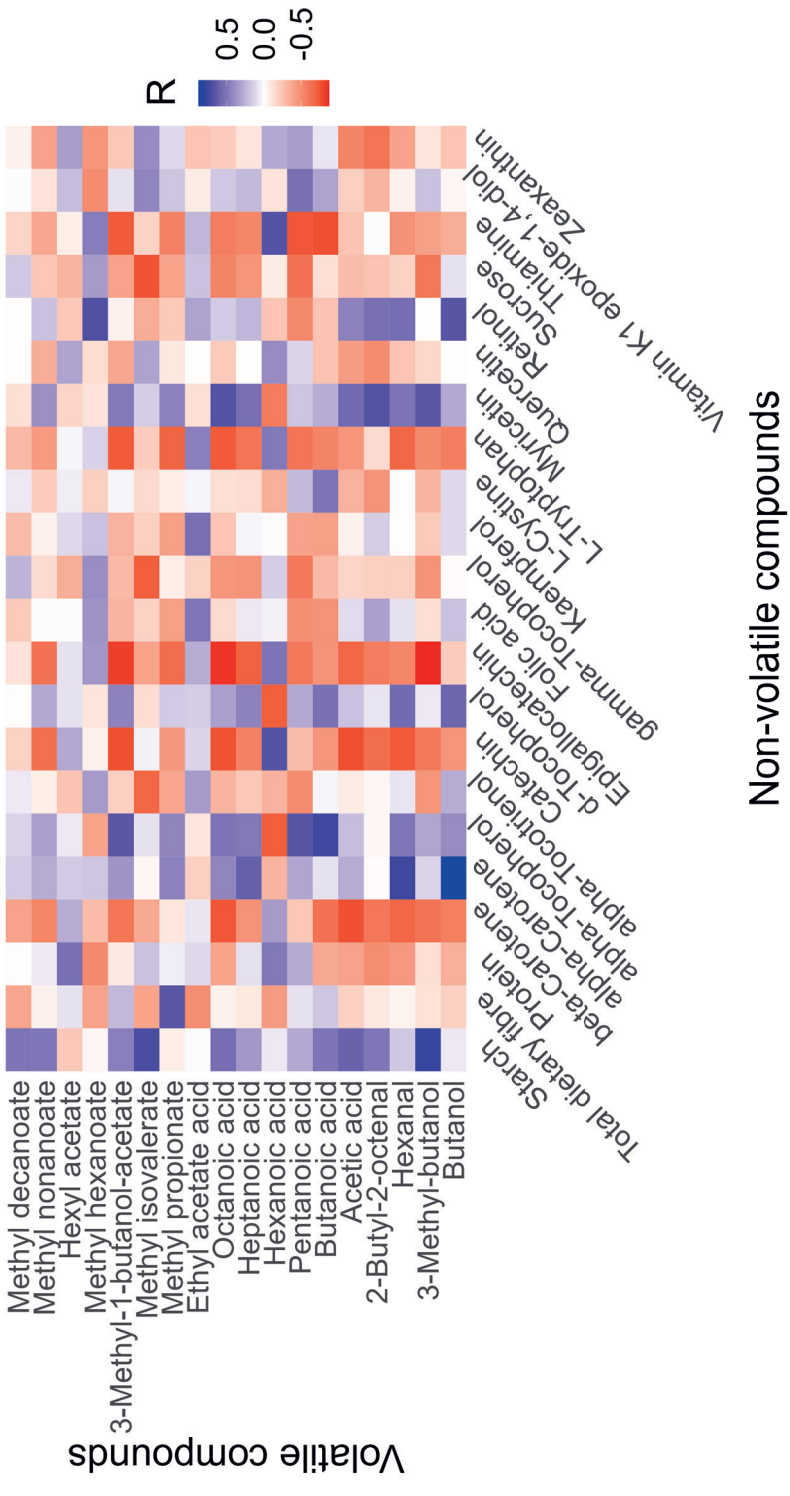


Figure 4.5. The correlation heatmap between volatiles along the vertical axis and non-volatiles along the horizontal axis. (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

4.4. Conclusions

The HS-SPME-GC-MS and DART-HRMS techniques enabled examination of banana pulp recording volatile and non-volatile compounds to explore the critical role of growing conditions. A total of 18 volatiles were identified in banana pulp powder and belong to the following main chemical groups: esters, alcohols, organic acids, and aldehydes. ANOVA results indicated the contents of volatile compounds showed significant differences among different farms. The PCA biplots indicated that banana volatiles can reflect the samples distributions according to their geographical origins and production systems to some extent. The main contribution of volatile compounds to separate the growing conditions are 3-methyl-butanol, 2-butyl-2-octenal, butanol, 3-methyl-1-butanol-acetate, pentanoic acid, methyl decanoate, and hexanoic acid. Furthermore, the non-volatile compounds of bananas were also analysed. The same statistical analysis applied to the non-volatile compounds of bananas showed an unclear distinction clustering for geographical origins but a clear distinction for organic and conventional systems in the PCA biplots. The non-volatiles such as folic acid, zeaxanthin, γ-tocopherol, and L-tryptophan are considered in this study to be the main drivers for separating the organic and conventional production systems. Moreover, significant correlations between volatile compounds such as 3-methyl-1-butanol-acetate, 3-methyl-butanol, and non-volatiles such as starch, total dietary fibre, and folic acid were observed in the banana pulp powder. Although this study was limited to the number of banana samples per farm and the diversity of geographical origins, the effects of growing conditions on volatile and non-volatile compounds of bananas were demonstrated. In conclusion, the possibility of a linkage between banana compositions and growing conditions established by volatile and non-volatile profiles seems promising, which provides potential insights to prevent food fraud in banana supply chains.

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CHAPTER 5

The relations between hyperspectral images of bananas (*Musa* spp.) from different countries, their compositional traits, and growing conditions

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Wang, Z., Erasmus, S. W., Liu, X., & van Ruth, S. M. (2020). Study on the relations between hyperspectral images of bananas (*Musa* spp.) from different countries, their compositional traits, and growing conditions. *Sensors, 20*(20), 5793.

Abstract

Bananas are some of the most popular fruits around the world. However, there is limited research that explores hyperspectral imaging of bananas and its relationship with the chemical composition and growing conditions. In this study, the relations that exist between the visible near-infrared hyperspectral reflectance imaging data in the 400–1000 nm range of the bananas collected from different countries, the compositional traits, and local growing conditions (altitude, temperature, and rainfall) and production management (organic/conventional) were explored. The main compositional traits included moisture, starch, dietary fibre, protein, carotene content, and the CIE L*a*b* colour values were also determined. Principal component analysis showed the preliminary separation of bananas from different geographical origins and production systems. The compositional and spectral data revealed positively and negatively moderate correlations (r around ± 0.50, $p < 0.05$) between the carotene, starch content, and colour values (a*, b*) on the one hand and the wavelength ranges 405–525 nm, 615–645 nm, 885–985 nm on the other hand. Since the variation in composition and colour values were related to rainfall and temperature, the spectral information is likely also influenced by the growing conditions. The results could be useful to the industry for the improvement of banana quality and traceability.

Key words

Correlation analysis; Geographical origin; Organic; VIS-NIR hyperspectral fingerprints

5.1. Introduction

It has been shown that eating bananas provides health benefits in respect of hypertension, cancer, diabetes, and depression (Pereira & Maraschin, 2015), given their composition of several essential nutrients, such as potassium, vitamins, carotenoids, manganese, fibre, and dopamine (Singh, Singh, Kaur, & Singh, 2016). Bananas can also be easily added to a regular diet by simply eating fresh fruits or by adding them to other foods (i.e., yoghurt or smoothies). Therefore, bananas are globally frequently consumed fresh fruit. The health benefits associated with the consumption of bananas and related processed products are highly correlated with the essential nutritional contents (Aurore, Parfait, & Fahrasmane, 2009; Sulaiman et al., 2011), while the quality and composition of bananas are affected by their origin and related growing conditions.

Previous studies have demonstrated the effect of origin and related growing conditions on the composition of fruits. For example, Margraf, Santos, de Andrade, van Ruth, and Granato (2016) reported that the geographical origin can influence the total soluble solids of Brazilian grape juices, while research on olive fruits from five different Turkish regions showed that environmental factors can potentially influence the maturity of the fruits and their phenolic fractions (Ghorbal et al., 2018). Different geographical parameters, including annual average temperature, altitude, and precipitation, can affect the physicochemical properties of fruits. For instance, the polyphenol content of pomegranate juice is highly influenced by temperature in the maturity period and growing latitude (Li et al., 2015). In addition, constant rising temperatures reduce the freshness and change the ruby colour of Bordeaux wines (produced from grapes) by changing the content and metabolism of flavonoids (Drappier, Thibon, Rabot, & Geny Denis, 2019). For bananas, there are only a few papers that have reported the effects of growing conditions on their composition (Fu et al., 2018; Stewart & Ahmed, 2020).

Banana export is one of the important economic pillars for many tropical and subtropical countries, such as the Philippines and Ecuador (FAO, 2021); banana exports reached a record high of 19.2 million tonnes in 2018. Therefore, traceability of the geographical origin of bananas is quite important for protecting the integrity of the banana supply chain and helps to prevent unfair competition – especially in cases

where a premium price is linked to a certain production system or origin. Hence, considering the popularity, health benefits, and economic importance of bananas, exploring the relationship between banana quality and growing conditions is necessary. A way to address banana geographical traceability is through data analysis and discrimination methods.

In the last few decades, important technologies have been identified that can be successfully applied to food authenticity and traceability research, such as liquid chromatography–mass spectrometry (LC-MS), nuclear magnetic resonance spectroscopy (NMR), near-infrared (NIR) spectroscopy, and hyperspectral imaging (HSI) (Medina, Perestrelo, Silva, Pereira, & Câmara, 2019). HSI coupled with chemometrics has been widely used for studies on food quality and safety (Ma, Sun, Pu, Cheng, & Wei, 2019). In comparison with other spectroscopy techniques, spectral characteristics as well as spatial information can simultaneously be recorded in one-time scanning with a HSI camera (Xu & Gowen, 2020). HSI technology has recently gained wide recognition in the field of food safety. Several studies in the food fraud domain have been conducted using HSI because of its advantages, such as rapidness, accuracy, reproduction, and portability (Oliveira, Cruz‐Tirado, & Barbin, 2019). Recently, using HSI, Acierno, Fasciani, Kiani, Caligiani and van Ruth (2019) reported that cocoa bean samples from South America and Africa show reflectance differences in the NIR wavelength range. Sun, Lu, Mao, Jin, and Wu (2017) claimed that the HSI system can provide rapid rice origin identification with a model accuracy of about 92% from combined spectral, morphological, and textural features. HSI can reflect the correlation between food composition and the optimum spectrum with the help of chemometrics (Choi, Heo, Bae, Kim, & Moon, 2020). On the other hand, food composition is usually influenced by local growing conditions, such as climate, rainfall, organic or conventional production (Mditshwa, Magwaza, Tesfay, & Mbili, 2017). However, no research has been conducted using HSI to explore its relationship with growing conditions and the physicochemical composition of bananas.

Hence, this research chapter aims to elucidate the relationships between hyperspectral imaging data of bananas (*Musa* spp.) from different countries (Dominican Republic, Ecuador, Colombia, Costa Rica, Panama, and Peru) using a portable HSI system in the visible (VIS) and NIR range (400–1000 nm) and

compositional traits and the bananas' growing conditions. The underlying factors were examined by correlation of the spectral data with compositional data of the bananas (physicochemical properties: carotene, starch, water, protein content, and colour values) and their growing conditions (altitude, temperature, rainfall, and production system). The relationship between datasets was established using chemometrics. The importance of this research relates to bridging the knowledge gap between the interaction of the environment with a food product, and how these extrinsic/environmental factors can influence the intrinsic quality characteristics. It also helps to establish a potential tool by which these characteristics can be measured and offers a way to trace the geographical origin of a food product.

5.2. Materials and methods

5.2.1. Sample collection and preparation

As shown in Figure 5.1, the research area was distributed in the main banana-producing areas from 19°42′3.0″ N to 4°51′47.3″ S and from 83°56′27.21″ W to 71°02′ 12.2″ W. Ten farms were selected as sampling sites; they were located in Central and South America, including Dominican Republic (two farms: DR1 and DR2), Ecuador (two farms: EC1 and EC2), Colombia (one farm: CO1), Costa Rica (three farms: CR1, CR2, and CR3), Panama (one farm: PA1) and Peru (one farm: PE1). Although these regions are known for high temperature and abundant precipitation, differences in the growing condition of bananas still remain due to altitude, latitude, annual precipitation, average temperature, and the amount of daily sunshine. To limit external variation and ensure that the only variation came from the growing conditions, bananas of the same variety, namely *Cavendish Williams*, were collected in their early maturity phenological stage in the same harvest year of 2018. Ten banana bunches were randomly selected from each farm. Only the bananas from the top position of bunches were selected to obtain high-quality samples. The banana bunches were packaged in pre-cleaned polyethylene bags and transported to Wageningen University & Research by courier in a low-temperature environment (11–13 °C). (Table 5.1). Two banana fingers from each bunch (per farm) were selected randomly to represent one sample of the specific farm. Ten banana samples were collected from each farm: totalling 100 samples for the study. The peel and pulp of the bananas were separated, freeze-dried, and

pulverized into a fine powder prior to the HSI image acquisition (Section 5.2.3) and compositional analysis (Sections 5.2.4 and 5.2.5).

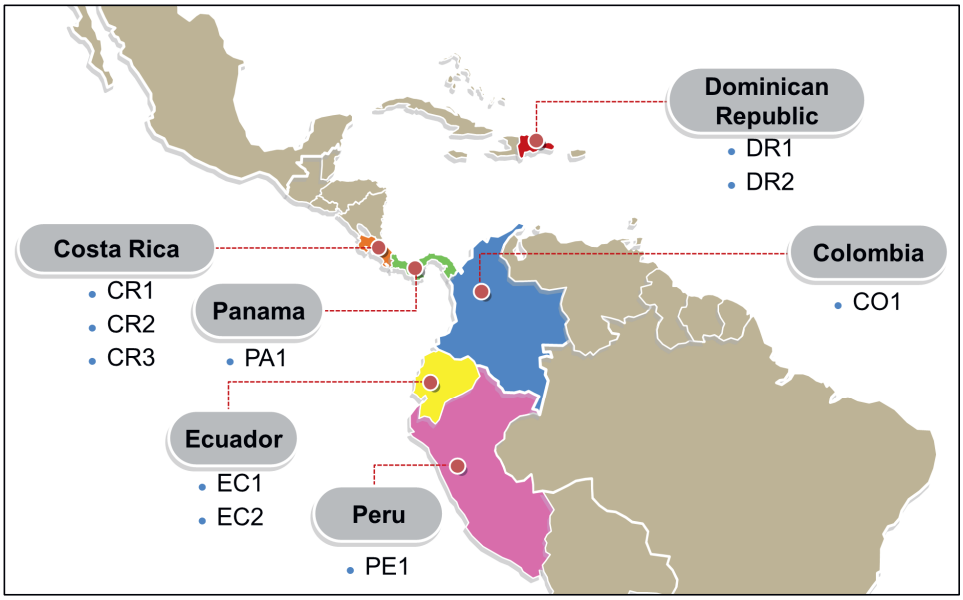


Figure 5.1. The sampling sites of bananas. (CO: Colombia; CR: Costa Rica; DR: Dominican Republic; EC: Ecuador; PA: Panama; PE: Peru; numbers refer to individual farms in a country). Map modified based on YourFreeTemplates.com (Latin America map free templates, 2016) and shared according to CC BY-ND 4.0. (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

5.2.2. Geographical data

The Global Positioning System (GPS) coordinates of the various sampling sites were provided by the local farms. The growing condition of bananas, including altitude according to GPS, monthly mean temperature and annual rainfall of harvest year were collected using Google Earth and public databases CRU TS4.04 (Climatic Research Unit (CRU) Time-Series (TS) version 4.04 of high-resolution gridded data of month-by-month variation in climate) (Harris, I.C.; Jones, 2020) (Table 5.1).

Table 5.1. The number of banana samples (pulp and peel) collected per country as well as the associated growing conditions.

Country	Farm code	Pulp	Peel	Production system	Altitude (m)	Monthly mean temperature (°C)	Annual rainfall (mm/year)
Colombia	CO1	10	10	Conventional	66	23.2	1837
Costa Rica	CR1	10	10	Conventional	726	23.4	2857
	CR2	10	10	Conventional	47	24.4	5014
	CR3	10	10	Conventional	24	26.3	4378
Dominican Republic	DR1	10	10	Organic	65	26.7	925
	DR2	10	10	Organic	27	26.7	925
Ecuador	EC1	10	10	Organic	32	22.9	1511
	EC2	10	10	Conventional	22	26.5	843
Panama	PA1	10	10	Conventional	10	19.7	3679
Peru	PE1	10	10	Organic	40	24.1	200

5.2.3. Hyperspectral imaging

The schematic diagram of the hyperspectral system set is shown in Figure 5.2. The hyperspectral images of banana pulp and peel samples were acquired using a Specim IQ hyperspectral system (Specim, Spectral Imaging Ltd., Finland) coupled with six 50 Watt halogen lamps (Philips, The Netherlands). The Specim IQ is a portable hyperspectral camera that consists of a spectral camera (CMOS technology), a viewfinder camera (5 Mpix), a focus camera and a scanner with a motor for optics movement. The focal length of the camera is 21 mm and the effective resolution of the CMOS camera is 512 × 512 pixels. Banana pulp and peel samples were placed into a plastic container (20 × 30 cm) and then individually placed on a black plate. The distance between the samples and the camera was set to 45 cm. A white Teflon panel was initially captured and used as a white reference prior to the actual measurement. Individual sample images were acquired within 10 ms integration time in a spectral range of 400–1000 nm at 7 nm resolution in line-scan mode (Behmann et al., 2018). After the full image was scanned, raw data were recorded as separated data and corrected automatically by white and black references by the camera. The subsequent spectral extraction and multivariate data analysis were conducted by ENVI image analysis software (Harris Geospatial Solutions Inc, Colorado, USA) and MATLAB R2018b (The MathWorks Inc, California, USA) (Park & Lu, 2015).

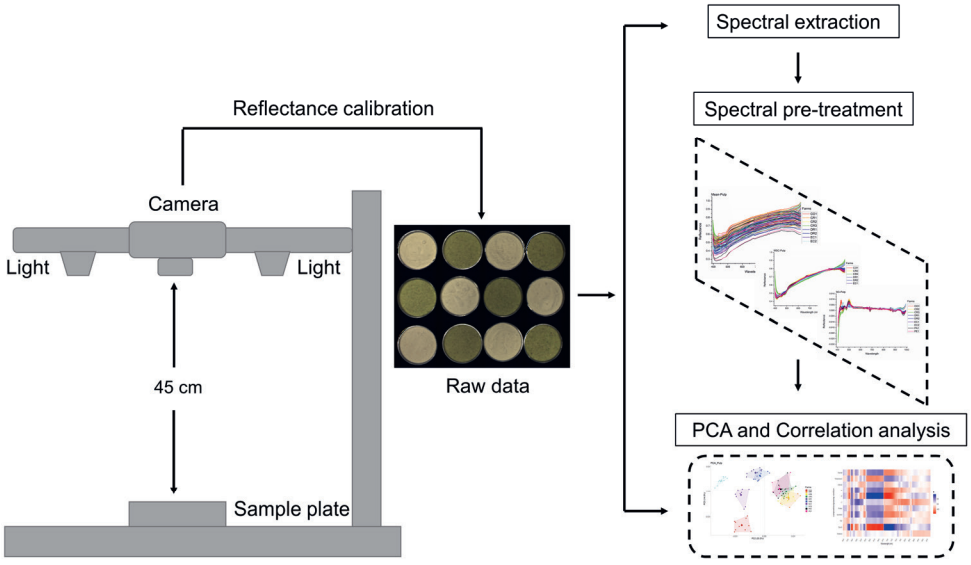


Figure 5.2. The schematic diagram of the hyperspectral system setup and steps for analysing hyperspectral images.

5.2.4. Determination of moisture, starch, total dietary fibre, protein, and carotene contents

The moisture content of fresh banana pulp and peel samples were calculated by recording the weight difference before and after freeze-drying, which is expressed as g/100g (wet weight). The following compositions were expressed based on dry weight. Total starch, diet fibre contents of banana pulp and peel were determined by the Total Starch Assay Kit (AA/AMG) and Total Dietary Fibre Assay Kit (Megazyme Ltd., Ireland) (Srichuwong et al., 2017). The protein contents were determined by Flash EA 1112 Protein analyser (Thermo Fisher Scientific, USA) using the Dumas combustion method (Jung et al., 2003). The carotene content was determined using a high-performance liquid chromatography (HPLC) method (Ayustaningwarno et al., 2020). For this HPLC method, the carotenes were extracted from the banana pulp and peel samples three times using a hexane and tetrahydrofuran solution until the pellet appeared colourless after centrifugation. The supernatant was then collected and evaporated using a vacuum evaporator. Finally, the extracted carotenes were dissolved in a sample buffer (MeOH–THF 1:1 + 0.01% BHT) and injected into the HPLC with a UV detector at 245.0 nm on an Agilent 1200 Infinity chromatograph. The mobile phase consisted of

acetonitrile (A), methanol (B), ethyl acetate (C) at ratios of 60:30:10 (*v: v: v*) and 0.1% triethylamine. With 20 µL injection volume, samples were separated on a Phenomenex Geminin C18 Column (5 µm, 250 mm × 4.6 mm) at 30 °C with a flow rate of 1.0 mL/min. A series of standard solutions were prepared by β-carotene (Sigma-Aldrich, USA). Each sample was prepared in triplicate.

5.2.5. Colour (L*a*b*) value measurements

Colour values of the banana pulp and peel samples were determined using a Colour Flex spectrophotometer (Hunter Associates Laboratory, Inc., Reston, USA) (Ashwar et al., 2016). The colour meter was first standardised using a green tile, after which the banana pulp and peel powder samples were weighed (2 mg) into cuvettes, and the L*, a*, and b* values measured. Each sample was measured three times to obtain an average colour reading.

5.2.6. Statistical analysis

5.2.6.1. Data processing of hyperspectral images

As shown in Figure 5.2, after acquiring the images, the region of interest (ROI) was selected using the ENVI 5.3 software (Harris Geospatial Solutions Inc, America.), and the full-pixel spectra of all banana pulp and peel samples were extracted in MATLAB R2018b. The spectral value of the average spectrum of each sample was collected to generate discrimination models of geographical origin. Prior to the development of the models, spectral pre-treatment of the raw data was performed using the multiplicative scatter correction (MSC) and Savitzky–Golay (SG) methods to reduce background noise and improve spectral resolution (Yin, Zhang, Zhu, Zhao, & He, 2017). After the spectral pre-treatment, principal component analysis (PCA) was performed for multivariate exploration of image data. Kendall correlation coefficients (r) for the spectral data, chemical composition, colour values, and the growing conditions of bananas were generated in a heatmap to show their relationships by R 3.5.3 software (R Foundation for Statistical Computing, Vienna, Austria).

5.2.6.2. Data analysis of banana compositions

The Shapiro–Wilk test was conducted to determine if the data was normally distributed (Shapiro & Wilk, 1965). Then, all the compositional data were further analysed and reported as mean values ± standard deviation. The significant level was determined by one-way analysis of variance (ANOVA) with Tukey's significant difference at a 5% significance level ($p < 0.05$). The relationship between chemical composition and growing conditions of bananas were evaluated by Kendall's correlation coefficients (r). All statistical analyses were performed using Unscrambler 10.5 (Camo Analytics, Norway) and R 3.5.3 (R Foundation for Statistical Computing, Austria) software.

5.3. Results and discussion

5.3.1. Explorative analysis of the spectral features, compositional traits of banana pulp, peel, and related growing conditions

The spectral profiles obtained from the hyperspectral images of banana pulp and peel samples are shown in Figure 5.3. Most of the samples show similar reflectance values in mean and MSC spectra. Two necessary spectral pre-processing methods (e.g., MSC and SG transformation) were used to remove certain patterns and noise among variables and to further explore spectral differences. Particularly, SG can correct the spectrum baseline intensity caused by different particle sizes (Gao, Li, Zhu, & He, 2013). The transformed spectra of pulp and peel are shown in Figure 5.3. The SG transformation showed considerably more variation in spectra to reflect the spectral differences of pulp and peel samples. Furthermore, PCA was used to explore the grouping of banana pulp and peel samples from different farms based on their spectral signatures after SG transformation. The groups in the PCA plots were labelled at farm level other than the country level as several farms originated from the same countries but were cultivated with different production systems (i.e., organic and conventional methods). Therefore, the sample grouping based on geographical origin and production system can be seen in the same PCA.

The banana pulp from different farms and production systems shared similar patterns of mean spectra (Figure 5.3a). However, there were differences in the position and intensity of the absorption peak. The pulp spectra had absorption peaks in the

following ranges: 400–500, 640–700, and 850–920 nm. The same is evident for the peel spectra. As shown in Figure 5.3b, the average spectra of the peel samples in the ranges 420–490 nm and 630–720 nm reflected obvious absorption peaks. The spectral differences could be caused by different geographical origins and growing conditions. For example, Sun et al. (2017) reported that all rice samples from four different regions showed characteristic absorption peaks in ranges of approximately 500–1000 nm. However, another side to consider is the evidence, as reported in recent papers, that growing conditions normally influence and cause differences in the compositional traits of foods (Margraf et al., 2016). Therefore, it is also of value to look further into the effects of growing conditions, which is specific per geographical origin, on the physicochemical composition of bananas. For the current study, the growing conditions of the banana farms are summarised in Table 5.1. Table 5.2 shows the chemical composition of the banana samples, including moisture, total starch, total dietary fibre (TDF), protein, β-carotene contents, and CIE L*a*b* values.

The data distribution of chemical and colour values was shown using violin plots in Figure A5.1. In brief, the chemical compositions and colour values of pulp and peel samples were different according to sampling sites. The differences could be due to the variation of local growing conditions. It has been reported that bananas harvested from a high altitude (300 m) usually have more aroma compounds and present a firmer texture than those harvested from a lower altitude (50 m) (Bugaud, Chillet, Beauté, & Dubois, 2006). The highest altitude (726 m) was observed in one of the farms (CR1) in Costa Rica. The lowest altitude (10 m) was reported for farm PA1 from Panama (Table 5.1). Correspondingly, the chemical analysis indicated that banana pulp from CR1 had significantly higher total fibre content than PA1 (Table 5.2). The different absorptions at 876 nm and 967 nm in the NIR region of the banana pulp samples are related to the C–H group of fibre and the O–H stretching vibration of starch. Absorptions at 871, 967, and 972 nm typically represent the dietary fibre (C–H), starch (O–H), and saccharide (O–H) components in peel, respectively (Workman & Weyer, 2007). Figure 5.2 shows the absorption differences in SG transformation spectra of pulp and peel in the above-mentioned wavelengths, which is linked to the starch and total dietary fibre contents (TDF) of the samples. Banana pulp and peel contained high amounts of starch and TDF (Table 5.2). For all the peel samples, the TDF contents are significantly higher than that of the pulp samples. This was expected as the peel is the

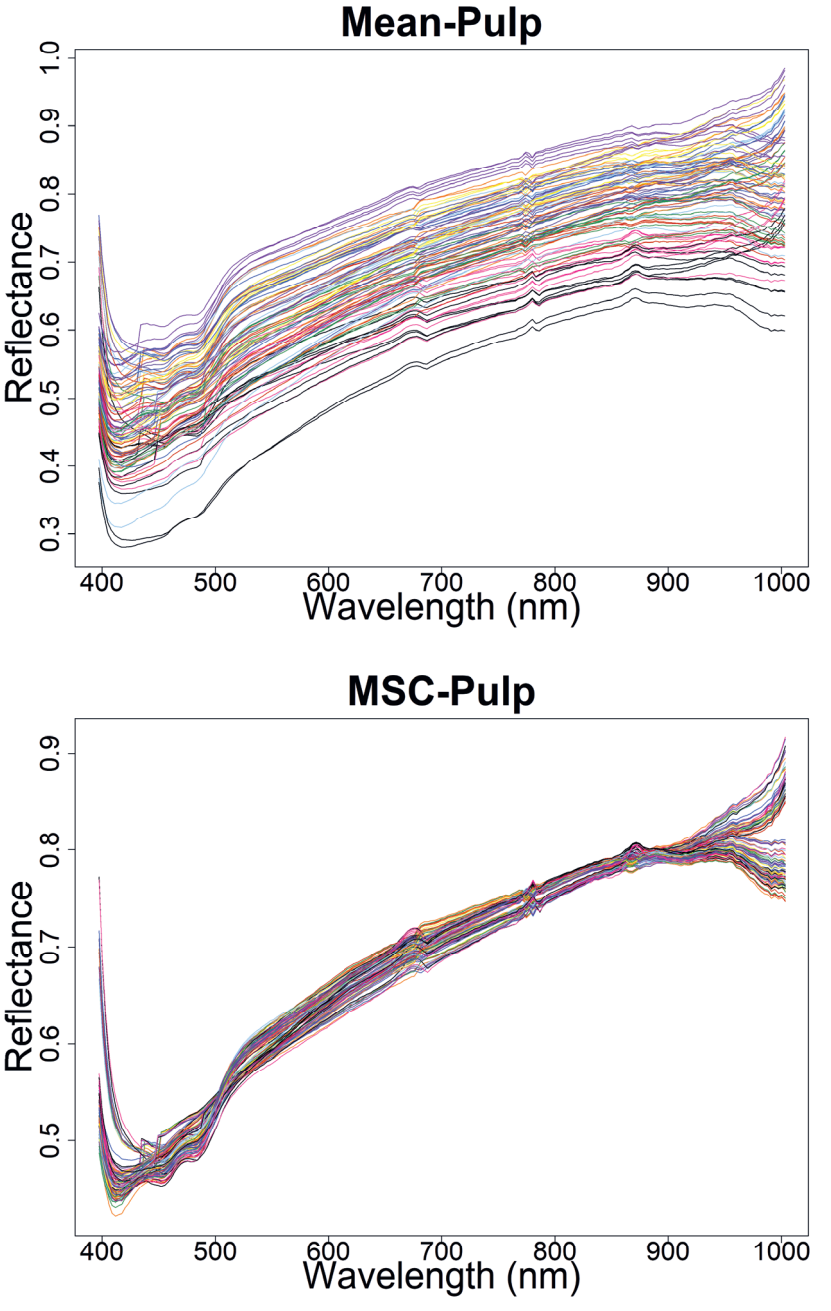
outer, fibrous layer of the fruit, while the softer/less fibrous part is the pulp. In the current study, the total starch content of banana pulp ranged from 28.8 ± 4.4 to 70.9 ± 0.4 per 100 g DW (dry weight). When compared to the other farms, the banana pulp from PE1 had the lowest starch content at 28.8 ± 4.4% and also a lower rainfall (200 mm/year). While for TDF, the peel samples from the PA1 farm had the highest TDF content (63.6 ± 6.0%) with a higher rainfall of 3679.3 mm/year. For CO1, DR1, EC2, their TDF contents were significantly lower than PA1. These TDF findings are consistent with recent research on the composition of bananas (Emaga et al., 2011; Pareek, 2016). These differences in composition could be due to rainfall as water is critical for the normal growth of bananas.

The values of the CIE L*a*b* colour coordinates were presented as mean ± standard deviation in Table 5.2 and Table 5.3. Higher b* values correspond to samples that appear more yellow, whereas with lower a* values the samples will appear greener. Similarly, the L* is for the lightness from black (lower/negative values) to white (higher/positive values). For the colour measurement of banana pulp and peel, the L* values and b* values showed a significant difference between most farms. In the visible spectral regions (350–780 nm) of banana pulp, wavelengths of 400–500 nm and 660–705 nm showed significant absorptions related to green and orange colours, which are consistent with the banana pulp colour values of ‘light yellow’ on the basis of the colour meter (b* value). Absorption at 455 nm and 460 nm indicates the presence of the carotenoids (Sun et al., 2019). As reported, bananas with yellow and orange pulp were rich in β-carotene (Bugaud, Daribo, & Dubois, 2007). As found in Table 5.2, the banana pulp samples from Farm CR2 and CR3 show increased absorption at 455 nm and 460 nm, and will likely be rich in β-carotene.

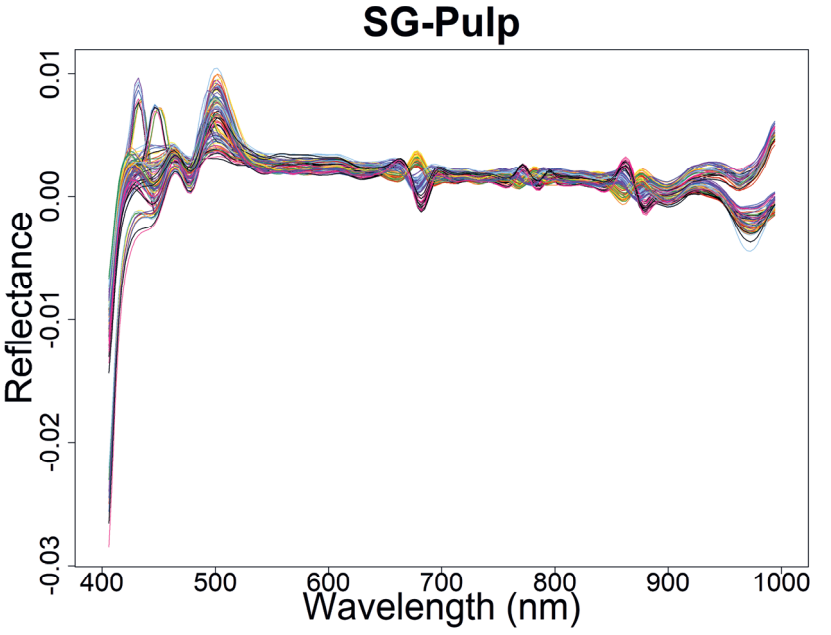
In Table 5.2 and Table 5.3, the content of β-carotene for pulp and peel showed significant differences between different countries, while the HSI spectral results were in line with these differences. For instance, the peel samples showed strong absorption in the 470–510 nm range, reflecting the green part of the peel, while absorption at 700 nm indicates a yellow-orange colour (b* value) for the peel. The variation in β-carotene content could be due to the growing conditions. As seen in Table 5.1, PA1 had the lowest monthly average temperature (19.7°C) in terms of the average temperature of all sampling farms, whereas the highest (26.7°C) was reported for DR1 and DR2 from the Dominican Republic. A similar trend is seen for the β-carotene content of these

samples, where banana pulp from PA1 had a higher β-carotene content (1.8 ± 0.2 μg/mg) than that of DR1 and DR2 (0.1 ± 0.1 μg/mg, respectively). Yang, Song, Fillmore, Pang, and Zhang (2011) found that high temperatures contribute to represses chlorophyll degradation and influences the colour of banana peels. Thus, this could indicate that different growing conditions could result in differences in β-carotene contents. However, it is important to note that temperature is not the only growing condition that can influence β-carotene content. For these farms, other conditions could likely have played a role. This was consistent with other studies about fruits, which indicated that temperatures, altitudes and rainfall are correlated with secondary metabolite synthesis such as β-carotene (Cardoso, Tomazini, Stringheta, Ribeiro, & Pinheiro-Sant'Ana, 2011; Reche et al., 2019). Therefore, the higher β-carotene content in CR2 and CR3 could be caused by higher rainfall. In the meantime, the high β-carotene content of PE1 could be due to organic production used in Peru as Bunea et al. (2012) reported that organic agriculture resulted in a higher β -carotene concentration in grape. However, there are also papers illustrating no evidence of nutritional superiority of the organically grown fruits (Cardoso et al., 2011). Yet, for the current study, there is not enough evidence to prove this effect and more research is needed for its verification. As for the β-carotene content in the peel samples, Colombia, Costa Rica, Peru, and Panama had significantly higher contents compared to those from the Dominican Republic and Ecuador (Table 5.3). The content differences in β-carotene according to geographical origins were similar to results reported for other food products, such as acerola cherry (Cardoso et al., 2011), jujube fruit (Reche et al., 2019), and milk (Brodziak, Król, Litwińczuk, & Barłowska, 2018).

(a)


Mean-Pulp
Reflectance
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
400
500
600
700
800
900
1000
Wavelength (nm)
MSC-Pulp
Reflectance
0.5
0.6
0.7
0.8
0.9
400
500
600
700
800
900
1000
Wavelength (nm)

(a) continued


SG-Pulp
Reflectance
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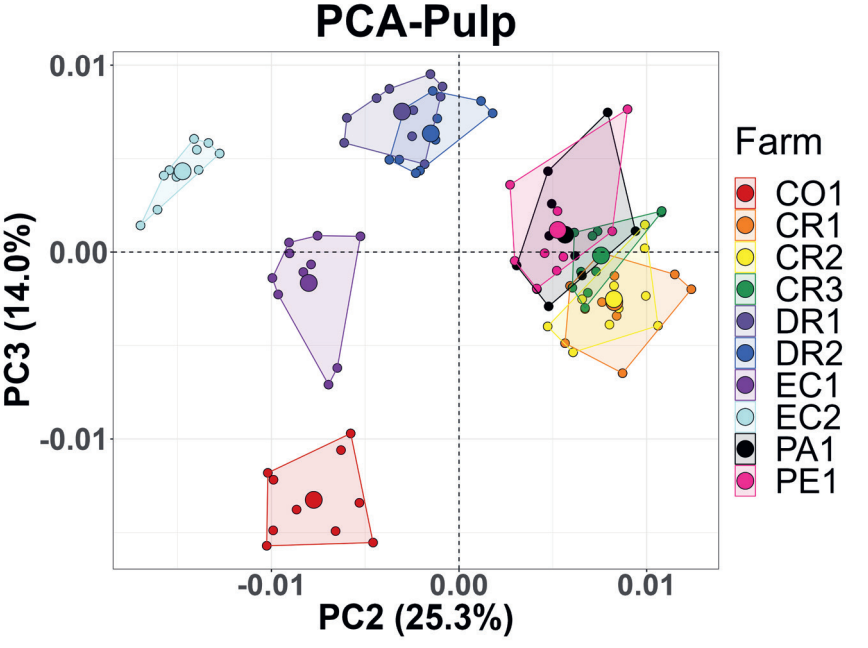
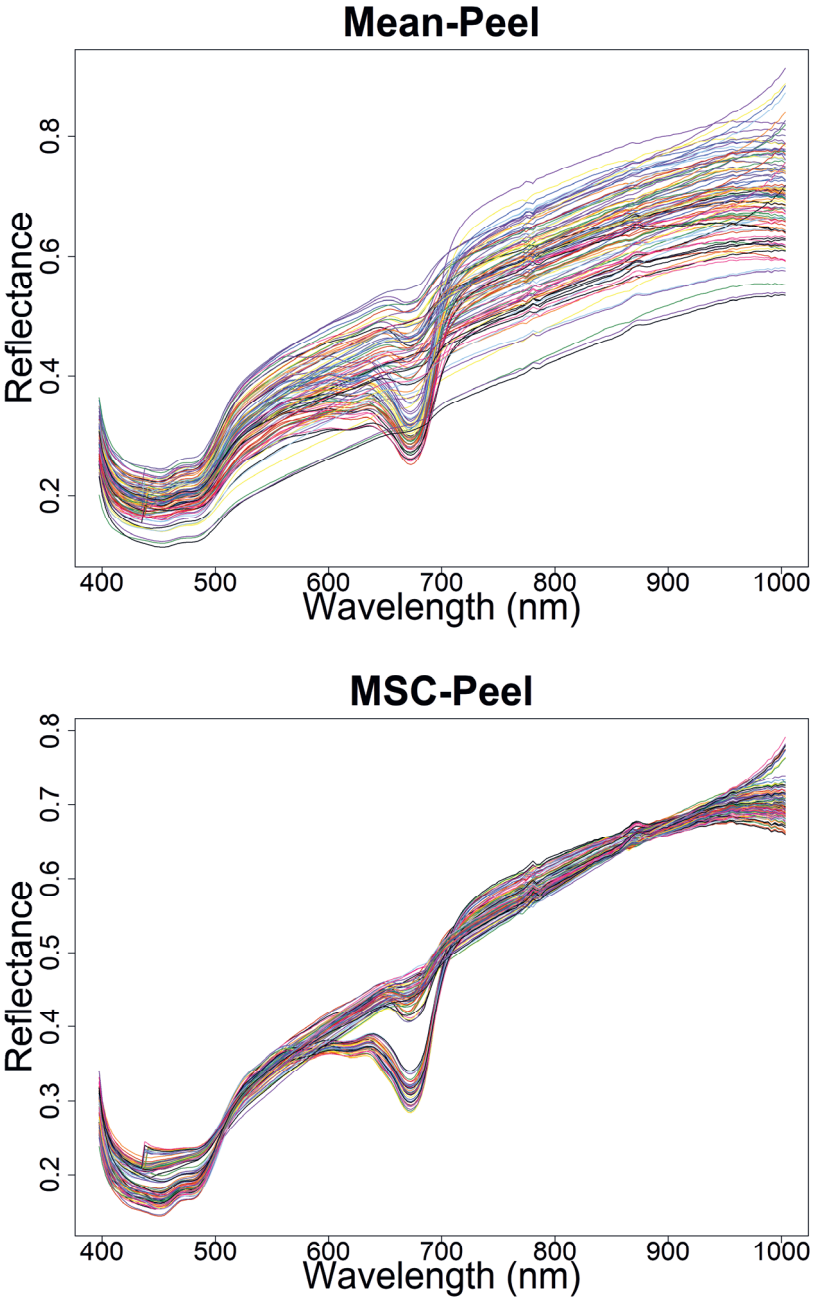
-0.01
-0.02
-0.03
400
500
600
700
800
900
1000
Wavelength (nm)


PCA-Pulp
PC3 (14.0%)
0.01
0.00
-0.01
-0.01
0.00
0.01
PC2 (25.3%)
Farm
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1

(b)


Mean-Peel
Reflectance
0.2
0.4
0.6
0.8
400
500
600
700
800
900
1000
Wavelength (nm)
MSC-Peel
Reflectance
0.2
0.3
0.4
0.5
0.6
0.7
0.8
400
500
600
700
800
900
1000
Wavelength (nm)

(b) Continued

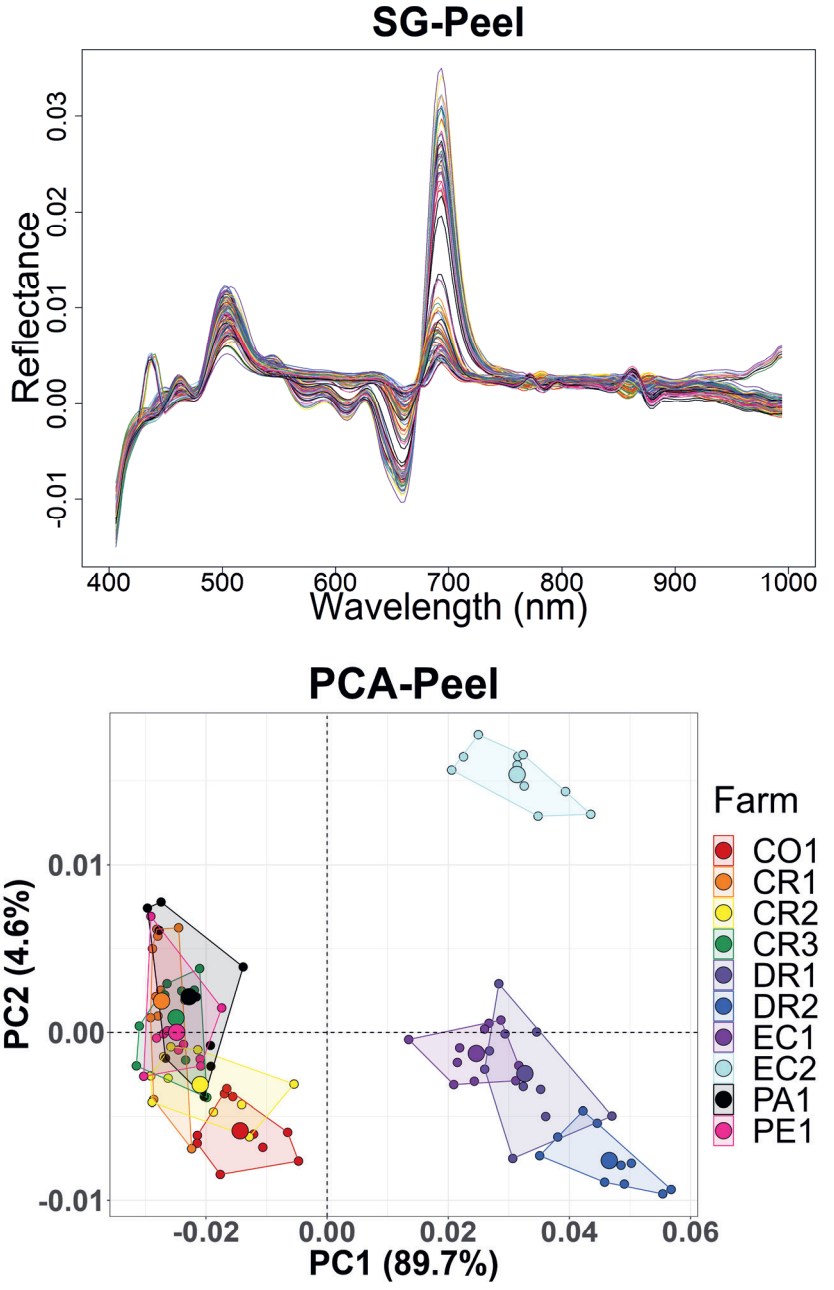


Figure 5.3. The mean reflectance (raw spectra), multiplicative scatter correction (MSC), Savitzky–Golay (SG) transformation spectra and principal component analysis (PCA)

plot (based on Hyperspectral Imaging data) of banana pulp (**a**) and peel (**b**) samples from different countries (CO: Colombia; CR: Costa Rica; DR: Dominican Republic; EC: Ecuador; PA: Panama; PE: Peru; (*n* = 10 for each farm)). (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

Differences in spectra due to the production system and growing conditions were also evident in the PCA plots (Figure 5.3). DR1 and DR2 were grouped separately from Peru (PE1), under the same organic production system, in the PCA plots of the pulp and peel samples (Figure 5.3). This grouping could be driven by the differences in altitude, temperature, rainfall, and production systems that led to a compositional variation of starch and TDF contents which results in differences in the characteristic spectral values. The two farms from Ecuador, EC1 (organic), and EC2 (conventional), also practiced different ways of cultivation, and grouped separately in the PCA plot of the pulp and peel spectra (Figure 5.3); further indicating that the production system could potentially be indicated by HSI. In light of this, Su and Sun (2016) found that the HSI conducted with PCA could easily separate organically and conventionally planted potatoes. Table 5.2 shows that EC1 and EC2 pulp samples had significant differences in TDF content. At the same time, the difference in rainfall of these two farms was also quite notably different: EC1 with 1511 mm/year and EC 2 with 843 mm/year.

A markable absorption around 995 nm that is likely related to the aromatic amines in pulp and peel spectral was identified, indicating that bananas often contain volatile substances and unique flavours (Boudhrioua, Giampaoli, & Bonazzi, 2003). For other compositional traits such as moisture and protein content, it is easy to recognise that banana peel has more moisture than pulp (~89% vs. ~74%, respectively) because the total starch content accounts for a large proportion of banana pulp. The pulp samples from DR1 had the highest moisture content (75.3 ± 5.9%), which is significantly higher than banana pulp samples from CR1, EC1, and EC2. Nguyen and Price (2007) found the moisture content (on a wet basis) to be 74.7 ± 1.3% for banana pulp from Australia, which reflects the similar values of the current study. For the moisture content of peel samples, the samples from the Dominican Republic (DR2) had the highest value, while the lowest content was reported in Ecuador (EC2). However, there are no significant differences between different groups, which means growing conditions did not have a great influence on the moisture content of bananas.

Lei et al. (2010) reported that bending vibration around 700 nm (N–H) was attributed to protein. In Figure 5.3, the variation of HSI spectra around 700 nm could be caused by different protein contents of pulp and peel. Generally, the banana peel samples have higher protein contents than the pulp samples (~5–6% vs. ~3–4%, respectively). PCA plots also showed that in comparison with the protein content of the pulp samples, the results revealed that banana peel had higher protein contents, such as 6.8 ± 0.7% for CR2. However, only a few farms showed significant differences in pulp and peel samples concerning the protein contents from all sampling countries. The DR1 bananas had considerably higher protein contents than bananas from other farms for pulp and reflected remarkable differences comparing peel samples from farm CR2 ($p < 0.05$). Table 5.1 showed that DR1 had quite a higher altitude, which could be the underlying mechanism. Mohapatra, Mishra, and Sutar (2010) also reported that the different content of banana protein could be caused by genomic mutation, altitude, and climate.

Not only the effects of geographical factors but also the effects of production systems were reflected in the PCA plots. As seen in Figure 5.2, three organic farms (DR1, DR2, and EC1), were separated from other conventional farms both based on HSI spectra of pulp and peel, which proved that the effects of different production systems can be observed in the hyperspectral results. Furthermore, compositional traits from Table 5.2 also showed that pulp from organic cultivation had higher ($p < 0.05$) contents of moisture, starch, and total dietary fibre and less ($p < 0.05$) β-carotene in comparison with conventional methods. The notably higher ($p < 0.05$) starch content also could be found in peel samples. However, one of the organic farms, namely PE1, cannot be separated from other conventional farms. This suggests that the difference in the HSI spectrum is due to the combined action of growing conditions. Lima and Vianello (2011) also pointed out that organic agriculture has great potential to increase the content of certain nutrients in food, but, obviously, there are more factors such as crop time, climate, soil characteristics, and environmental conditions that still make contributions.

Previous studies usually used the PCA plots to show the grouping of samples based on their different geographic locations (Margraf et al., 2016; Yin et al., 2017). However, our research shows that the potential differences in spectra are not only caused by geographical origin but are also very much related to the characteristic

growth conditions such as altitude, temperature, and rainfall. Even for the bananas collected from the neighbouring countries such as Colombia and Panama, the PCA plots showed that CO1 grouped separately from PA1 according to the pulps' spectral fingerprint as both farms have large differences in altitude and rainfall. For the peel samples, although the DR1 and EC1 farms had a quite long geographical distance between them, they were still grouped closely. This could be because both shared similar temperature, rainfall and were cultivated with a similar organic system. In view of this, Sun et al. (2019) also demonstrated that HSI conducted with PCA could address the difference between two shrimp groups from high- and low-salinity environments.

Table 5.2. The composition of banana pulp samples for the different countries (n = 10 for each farm).

Country	Farm code	Production system	Moisture # (g/100g)	Starch (g/100g)	Total dietary fibre (g/100g)	Protein (g/100g)	β-carotene (µg/mg)	L*	a*	b*
Colombia	CO1	Conventional	74.8 ^{ab} ± 1.7	31.4 ^{d} ± 5.5	17.6 ^{bd} ± 1.8	3.4 ^{ab} ± 0.3	0.2 ^{d} ± 0.1	83.8 ^{a} ± 0.6	0.4 ^{b} ± 0.2	12.1 ^{a} ± 0.5
Costa Rica	CR1	Conventional	72.4 ^{bc} ± 0.8	41.0 ^{cd} ± 4.1	19.4 ^{bc} ± 1.9	3.6 ^{ab} ± 0.3	0.2 ^{d} ± 0.2	83.5 ^{a} ± 0.7	0.7 ^{ab} ± 0.1	13.1 ^{b} ± 0.8
	CR2	Conventional	72.8 ^{ac} ± 0.7	36.4 ^{d} ± 1.5	17.5 ^{bd} ± 2.7	3.7 ^{ab} ± 0.3	3.0 ^{a} ± 0.4	84.1 ^{a} ± 0.4	0.7 ^{b} ± 0.1	12.5 ^{a} ± 0.8
	CR3	Conventional	73.0 ^{ac} ± 1.0	40.1 ^{cd} ± 5.2	17.0 ^{cd} ± 1.2	3.7 ^{ab} ± 0.2	2.5 ^{b} ± 0.3	81.5 ^{b} ± 0.8	0.7 ^{b} ± 0.1	11.4 ^{b} ± 0.4
Dominican Republic	DR1	Organic	75.3 ^{a} ± 5.9	62.0 ^{ab} ± 4.5	16.2 ^{d} ± 1.2	3.8 ^{a} ± 0.5	0.1 ^{d} ± 0.0	80.8 ^{bc} ± 0.7	1.1 ^{c} ± 0.1	11.8 ^{d} ± 0.5
	DR2	Organic	70.6 ^{cd} ± 1.0	70.9 ^{a} ± 0.4	19.7 ^{b} ± 1.75	3.5 ^{ab} ± 0.4	0.1 ^{d} ± 0.0	78.9 ^{d} ± 1.8	1.3 ^{d} ± 0.2	13.2 ^{b} ± 0.2
Ecuador	EC1	Organic	68.8 ^{d} ± 1.0	55.4 ^{b} ± 6.8	17.0 ^{cd} ± 2.0	3.4 ^{ab} ± 0.7	0.1 ^{d} ± 0.1	79.3 ^{d} ± 1.1	1.8 ^{c} ± 0.2	14.1 ^{c} ± 0.7
	EC2	Conventional	69.4 ^{d} ± 1.7	52.1 ^{bc} ± 6.2	19.1 ^{bc} ± 2.4	3.2 ^{ab} ± 0.1	0.0 ^{d} ± 0.0	79.7 ^{cd} ± 0.9	1.6 ^{c} ± 0.3	13.1 ^{c} ± 0.5
Panama	PA1	Conventional	72.6 ^{ac} ± 0.3	33.4 ^{d} ± 2.8	22.9 ^{a} ± 1.5	3.7 ^{ab} ± 0.3	1.8 ^{c} ± 0.2	84.1 ^{a} ± 0.8	0.5 ^{a} ± 0.1	11.7 ^{a} ± 0.3
Peru	PE1	Organic	74.0 ^{ab} ± 0.4	28.8 ^{d} ± 4.4	25.5 ^{a} ± 1.3	3.5 ^{ab} ±0.2	1.9 ^{c} ± 0.5	84.1 ^{a} ± 1.2	0.6 ^{ab} ± 0.2	10.9 ^{b} ± 0.3

$^{a–d}$ Different mean values in the same column with different superscript letters are significantly different ($p < 0.05$) according to Tukey's significant difference test; # Moisture content is based on wet weight, other compositions are based on freeze-dried (dry) weight.

Table 5.3. The composition of banana peel samples for the different countries (n = 10 for each farm).

Country	Farm code	Production system	Moisture # (g/100g%)	Starch (g/100g)	Total dietary fibre (g/100g)	Protein (g/100g)	β-carotene (µg/mg)	L*	a*	b*
Colombia	CO1	Conventional	89.2 ^{bd} ± 0.7	10.9 ^{c} ± 2.6	55.0 ^{bcd} ± 4.7	5.8 ^{bd} ± 0.7	1.7 ^{a} ± 0.1	67.1 ^{a} ± 1.4	3.2 ^{b} ± 0.5	25.7 ^{a} ± 0.7
Costa Rica	CR1	Conventional	88.9 ^{cd} ± 0.3	14.5 ^{c} ± 1.9	58.1 ^{ac} ± 8.9	6.6 ^{b} ± 0.5	1.5 ^{ac} ± 0.5	62.7 ^{bce} ± 3.6	3.9 ^{ab} ± 0.7	23.2 ^{b} ± 0.6
	CR2	Conventional	88.6 ^{cd} ± 0.9	15.7 ^{c} ± 1.0	51.1 ^{ce} ± 6.8	6.8 ^{b} ± 0.7	1.7 ^{a} ± 0.1	65.6 ^{ab} ± 1.0	3.4 ^{b} ± 0.3	24.8 ^{a} ± 0.6
	CR3	Conventional	89.5 ^{bc} ± 0.9	12.6 ^{c} ± 0.1	46.9 ^{ce} ± 4.5	6.2 ^{bc} ± 1.2	1.5 ^{ac} ± 0.1	63.8 ^{bcd} ± 1.7	3.4 ^{b} ± 0.2	23.0 ^{b} ± 1.1
Dominican Republic	DR1	Organic	90.4 ^{b} ± 2.0	30.3 ^{ab} ± 1.9	55.8 ^{bc} ± 2.9	5.3 ^{cd} ± 1.1	0.5 ^{c} ± 0.1	62.0 ^{ce} ± 2.2	0.6 ^{c} ± 0.7	19.6 ^{c} ± 1.2
	DR2	Organic	88.4 ^{cd} ± 0.8	31.7 ^{a} ± 1.7	55.9 ^{bc} ± 8.7	4.7 ^{d} ± 0.4	1.2 ^{ac} ± 0.2	64.4 ^{ac} ± 1.7	−1.5 ^{d} ± 0.6	22.6 ^{b} ± 0.6
Ecuador	EC1	Organic	88.1 ^{d} ± 0.6	25.6 ^{b} ± 3.2	47.1 ^{ce} ± 6.7	5.2 ^{cd} ± 0.5	0.6 ^{c} ± 0.1	60.9 ^{de} ± 0.8	1.1 ^{c} ± 0.5	21.1 ^{c} ± 0.6
	EC2	Conventional	88.0 ^{d} ± 0.6	32.7 ^{a} ± 1.9	47.9 ^{de} ± 9.0	5.1 ^{cd} ± 0.4	0.9 ^{c} ± 0.1	62.3 ^{ce} ± 1.1	0.7 ^{c} ± 0.5	21.3 ^{c} ± 0.7
Panama	PA1	Conventional	89.1 ^{bd} ± 0.3	12.8 ^{c} ± 1.8	63.6 ^{a} ± 6.0	6.1 ^{bc} ± 0.7	1.4 ^{ac} ± 0.3	60.1 ^{e} ± 5.4	4.6 ^{a} ± 0.7	25.0 ^{a} ± 1.6
Peru	PE1	Organic	88.5 ^{cd} ± 0.4	15.5 ^{c} ± 0.2	60.7 ^{ab} ± 7.8	4.8 ^{d} ± 0.4	1.2 ^{b} ± 0.1	65.0 ^{ac} ± 2.6	3.9 ^{ab} ± 0.7	22.9 ^{c} ± 1.5

$^{a–e}$ Different mean values in the same column with different superscript letters are significantly different ($p < 0.05$) according to Tukey's significant difference test; # Moisture content is based on wet weight, other compositions are based on freeze-dried (dry) weight.

5.3.2. Correlation of the HSI spectra, compositional traits, colour data, and growing conditions

In the last ten years, HSI technology has been widely used in food authenticity and fraud research, especially adulteration, agricultural product traceability, organic food identification and other related fields (Pu, Lin, & Sun, 2019). However, most articles mainly tend to use hyperspectral imaging techniques to accurately identify sample differences, but few works of literature report the causes of these differences. In the above parts of this paper, the chemical compositions and value databases from banana pulp and peel samples were obtained by a series of approaches. The growing conditions and HSI spectral data were acquired as well. To explore how HSI spectra relate to the differences in compositional traits and growing conditions, the spectral data were correlated with the compositional data, colour values, and growing conditions (Figure 5.4).

Figure 5.4a shows the correlation between the HSI spectra (Savitzky–Golay transformation) on the X axis and the compositional, colour, growing conditions on the Y axis. The correlation analysis was based on the characteristic peaks caused by certain compositions and colours of pulp and peel under HSI wavelengths 400–1000 nm (Yin et al., 2017). For the wavelength ranges 405–525 nm, 615–645 nm, and 885–985 nm, a relatively strong correlation was observed. The HSI spectra of the banana pulp samples (Figure 5.4a) also showed higher differences in these ranges according to different growing conditions. Compared with protein, TDF, moisture, the carotene, and starch contents showed a stronger correlation with HSI spectra in the 405–525 nm and 615–625 nm wavelength ranges. As the most abundant component of banana pulp, the moderate positive correlation ($r = 0.39–0.45$, $p < 0.05$) of starch with HSI indicates the potential of HSI to identify starch-rich foods. As reported in Section 3.1, absorptions at 876 nm and 967 nm are generally related to the C–H group of fibre and O–H stretching vibration of starch (Ørnholt Johansson, Frosch, & Munk Jørgensen, 2017), therefore moderate correlations (r values around 0.40, $p < 0.05$) of starch of pulp also were shown in the 885–985 nm range. Compared to banana pulp, weaker correlations of most of the traits and HSI exist for the peel samples. This is possibly due to the fact that banana pulp is more likely to reflect the influence of growing conditions on the composition and colour values. At the same time, the spectra of the banana peel and the PCA results also showed that the difference between the banana

peels is smaller than those for the pulps. Ultimately, HSI could reveal the difference in the composition of bananas and is a suitable tool to link the compositional traits to the growing conditions of bananas.

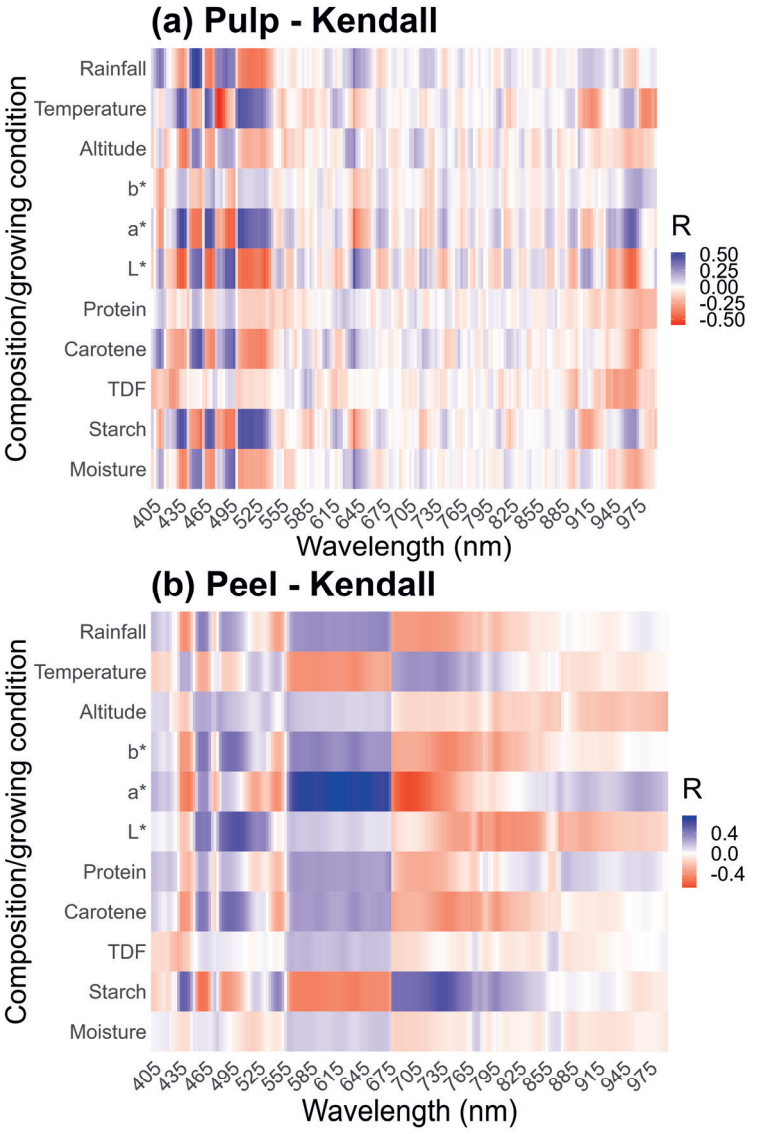


Figure 5.4. The heatmap to show the Kendall correlations (r) between wavelength, composition, and growing conditions for banana pulp (**a**) and peel (**b**). (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

5.3.3. Correlation between growing conditions and banana composition

Research studies on bananas have mainly reported the relationships between the climate and yield of bananas, while only a few papers have focused on the correlation of local growing conditions and banana compositional traits (Stewart & Ahmed, 2020). Researchers found that the contents of chemical compositions of plants the results of absorption, utilization, and metabolic activities (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). Other reported that the starch content of sweet potatoes was increased in high altitudes farms (Tumwegamire et al., 2011). Furthermore, the effects of different altitudes and temperatures on moisture content, reducing sugars, crude fibre as well as colour were studied in *yacon* tuberous roots (Silva, Lima, Oliveira, Teixeira, & Machado, 2018). However, the correlations of growing conditions and food compositions were still scarcely reported in the literature. To understand the effects of growing conditions on the composition and colour variation of the bananas, correlations between those datasets were investigated by Kendall's correlation coefficients (r).

In Figure 5.5, The coefficients and the significant correlations are shown from red (negative) to blue (positive) ($p < 0.05$), while the insignificant correlations are marked as blank. There are few publications reporting on correlations between the growing condition of banana and the composition. It was reported that the harvest season of the banana field will affect the firmness of the banana fruit (Fu et al., 2018). Apart from this, some research studies reported that a quite strong correlation (r = 0.62–0.94) was noted between rainfall levels and fruit firmness (Ornelas Paz et al., 2018). In the review, the cited research showed that the relationship of climate differences and fruit quality, the vitamin content, phenolic compound content, and micronutrients of fruits are highly affected by temperature change, water availability, and other geographical variables (Stewart & Ahmed, 2020).

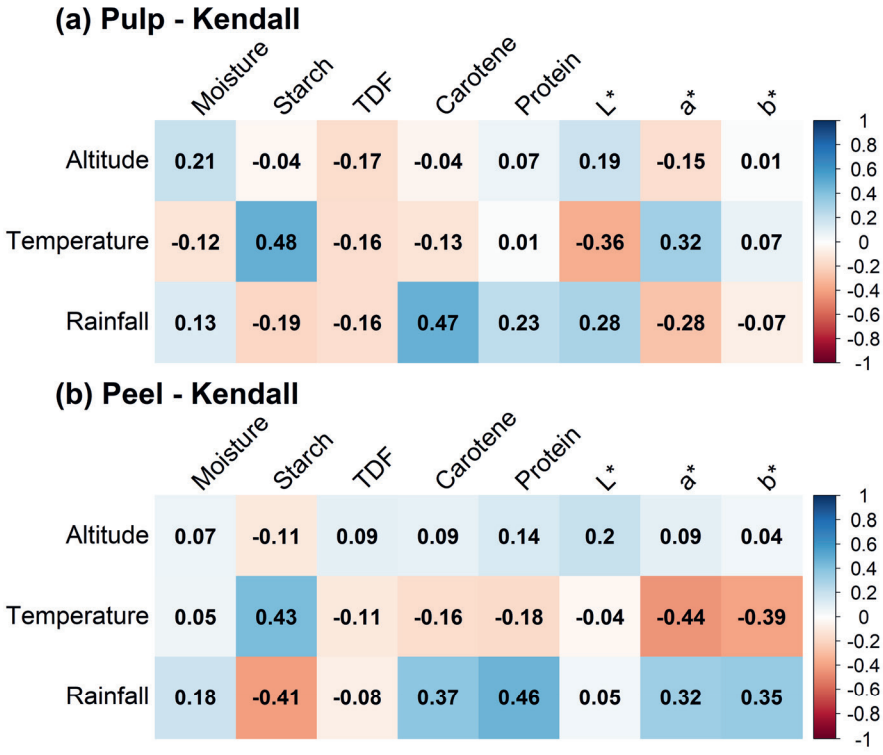


Figure 5.5. The heatmap to show the Kendall correlation (r) between growing condition and composition traits of banana pulp (**a**) and peel (**b**). (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

For the pulp samples, mostly positive correlations were found between temperature, rainfall, chemical composition, and colour values (Figure 5.5a). The colour index a* had a moderate and slightly positive correlation with temperature ($r = 0.32$, $p < 0.05$), while the L* factor correlated negatively with temperature. For the former, the positive correlation means that with increasing temperature an increase in a* values (more red and less green colour) can be expected. This finding coincides with the fact that a higher temperature is helpful to the growth of bananas and promotes the pulp to change from white to light yellow. This also explains why the L* value is positively correlated with temperature. Because L* stands for lightness of samples, the lightness of pulp will be decreased in the colour transformation during growth. Growing

conditions such as rainfall resulted in a moderate positive correlation ($r = 0.47$, $p < 0.05$) with carotene content for the banana pulp. Therefore, higher carotene contents can be expected for bananas grown in regions with more extensive rainfall. In our research, the banana pulp from CR2 had the highest carotene content, also the highest annual rainfall is reported on this farm. Most of the chemical and colour values were only slightly influenced by altitude, indicating that the effects of temperature and rainfall were higher than those of altitude.

In contrast to the pulp samples, temperature largely showed significant negative correlations with most of the chemical components and colour values of the peel samples. The temperature had a slight negative correlation with the protein content of peel samples ($r = -0.18$, $p < 0.05$), which could be due to the effect of temperature on banana metabolism. Similar correlations were determined for the protein contents of salmon fillets. Ørnholt-Johansson et al. (2017) reported that a negative relationship exists between the protein content of salmon and sea temperature.

Particularly, the temperature had a negative correlation with the carotene content ($r = -0.16$, $p < 0.05$), which is consistent with the findings in carrot research (Sulaeman et al., 2001). As was found in Section 5.3.1, the banana pulp farm PA1 within high temperature showed a higher β-carotene content than that of DR1 and DR2 within lower temperatures. A similar study about the influences of temperature on the colour of bananas also reported that a high temperature could decrease the yellow colour of bananas (Yang et al., 2011). A negative correlation between temperature and b* value ($r = -0.39$, $p < 0.05$) was observed, whereas the b* value reflects the yellow colour of banana peel. A moderate positive correlation was observed between rainfall and the b* colour value ($r = 0.35$, $p < 0.05$); hence, higher rainfall could enhance the banana peel colour as an increase in b* value will result in a more intense yellow colour of the peel. As reported in Section 5.3.1, farm PE1 with the lowest rainfall showed the lowest b* value compared with other farms ($p < 0.05$). From Figure 5.5b, the same correlation trends were observed between the results of carotene content and colour indexes of the peel samples. The impact of growing conditions on the fruit's compositions is due to the effect of temperature and rainfall affecting the metabolism of nutrients in fruits (Sim et al., 2017).

These results demonstrate that different growing conditions had certain effects on the chemical compositions and colour values of the banana pulp and peel samples. Climatic factors, such as annual average precipitation, relative humidity, and average temperature, have also been reported to have either positive or negative effects on the metabolites of safflower (*Flos Carthami*)(Cao et al., 2019). The different altitudes were also reported to have an influence on the organic acids and aroma volatile attributes of pomegranate fruit (Mphahlele, Caleb, Fawole, & Opara, 2016). Similarly, the CIE L*a*b* colour spaces are useful for the identification of honey types from different geographical locations (Tuberoso et al., 2014). When discussing food authenticity, it is vital to consider the growing conditions as food quality, flavour, and economic value often benefit from special geographical conditions.

5.4. Conclusions

The results of this chapter demonstrate that banana pulp and peel of fruits from different geographical origins and production systems have unique spectral fingerprints that can be linked to their chemical composition and associated growing conditions. PCA revealed that the spectral range from 400–1000 nm is an effective range to show the differences in geographical origin and production system of bananas using a portable HSI system. The combined effects of rainfall, temperature, and altitude are likely some of the main causes for the variation in the carotene, starch, and dietary fibre contents. The colour values are more related to altitude and temperature at the geographical locations. The wavelength ranges 405–525 nm, 615–645 nm, and 885–985 nm of HSI were significantly correlated (r values around ± 0.50, $p < 0.05$) with the carotene content, starch content, a*, and b* colour values. Indirectly, the differences in HSI spectral data could reflect the effects of rainfall, temperatures, and altitudes (growing conditions) on compositional traits of bananas. These findings help to better understand the effects of growing conditions on the colour differences and the variation in the composition of bananas.

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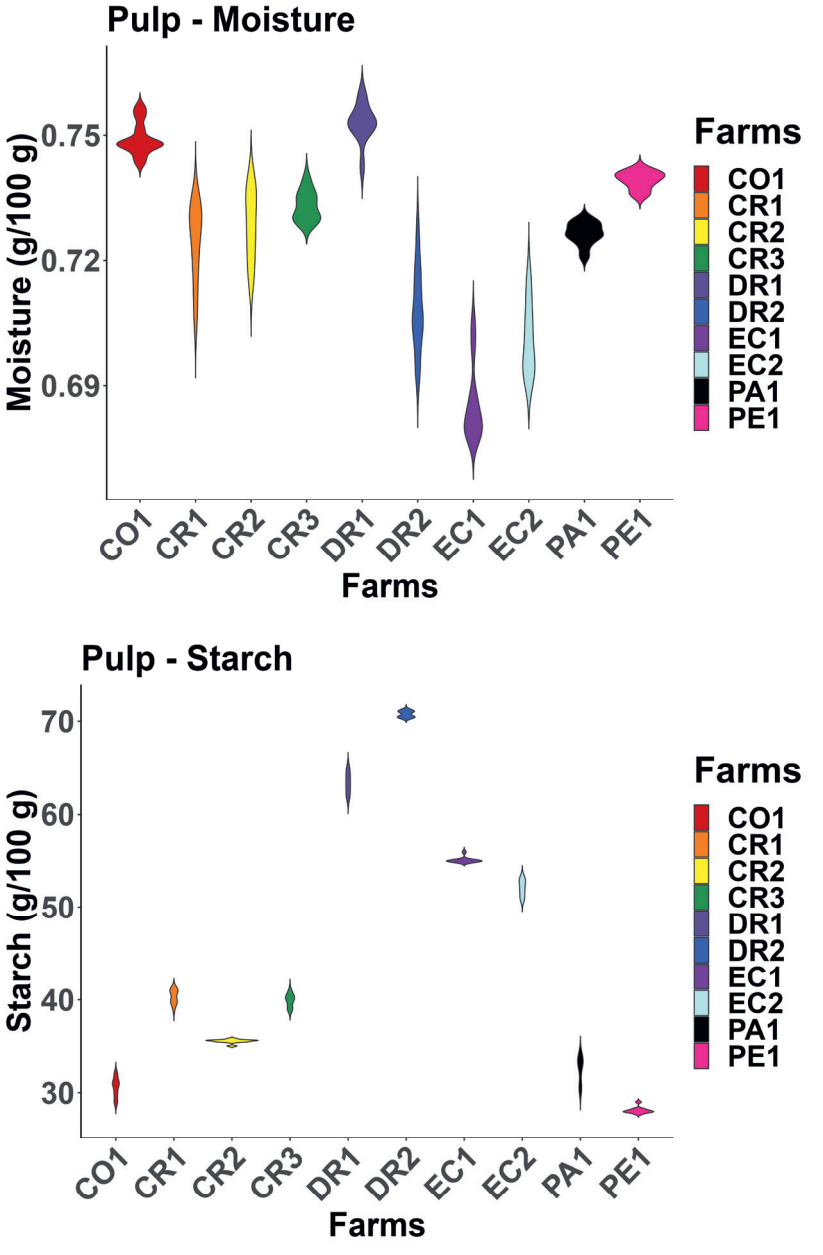
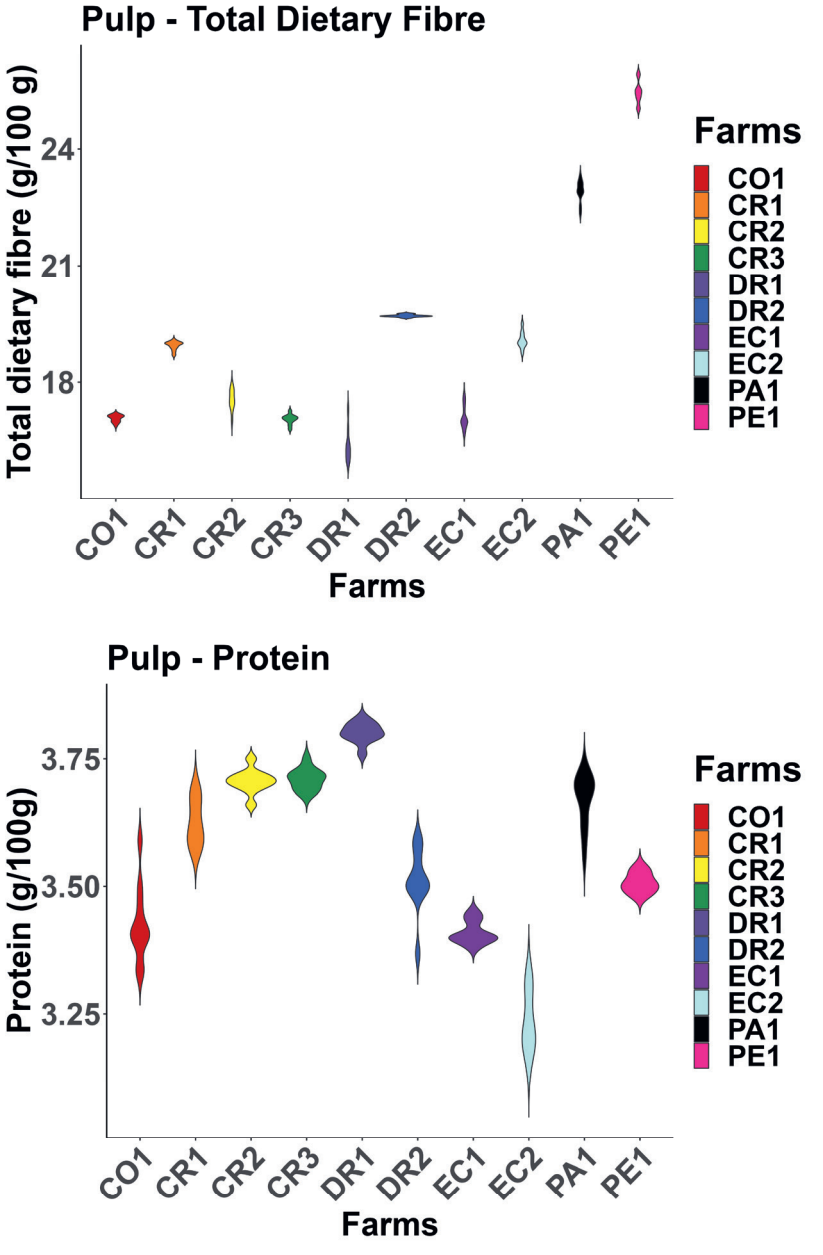
Appendix C.

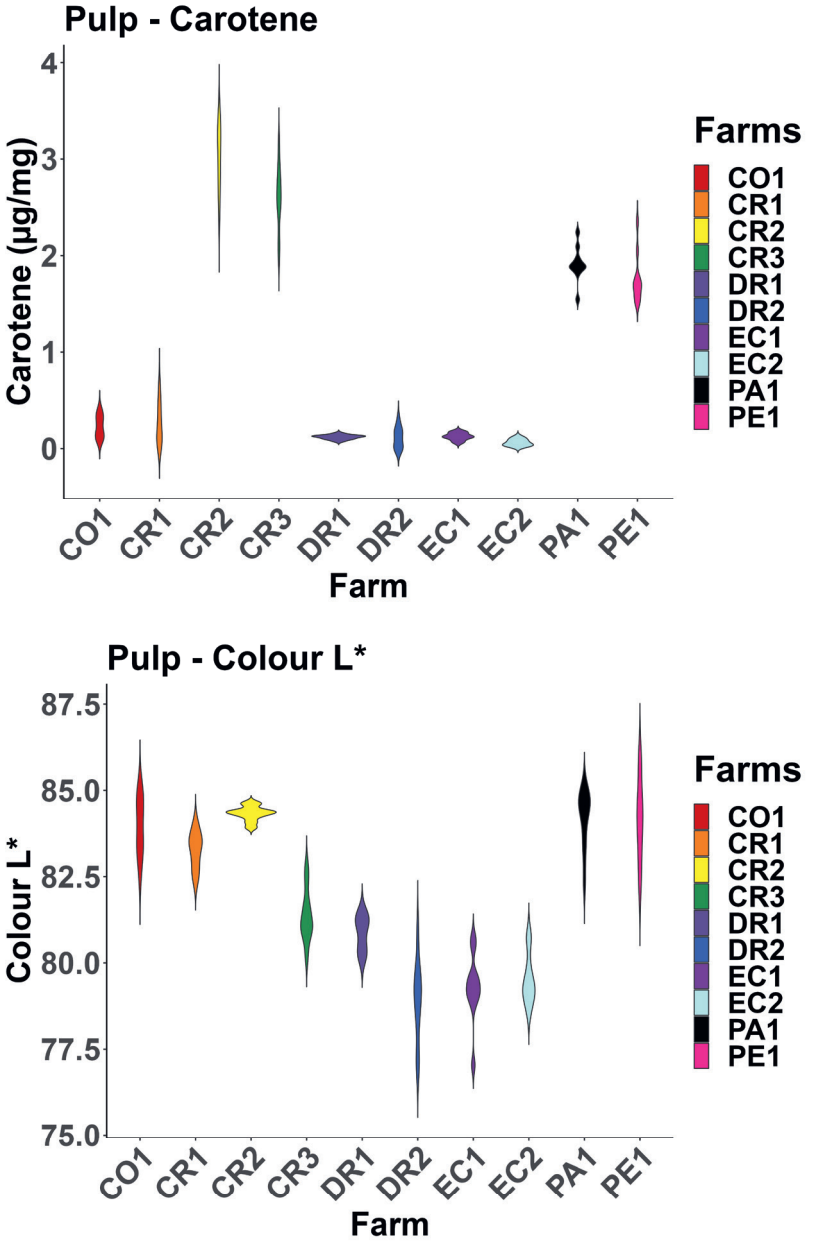
(a)


Pulp - Moisture
Moisture (g/100 g)
0.75
0.72
0.69
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Farms
Farms
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Pulp - Starch
Starch (g/100 g)
70
60
50
40
30
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Farms
Farms
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1

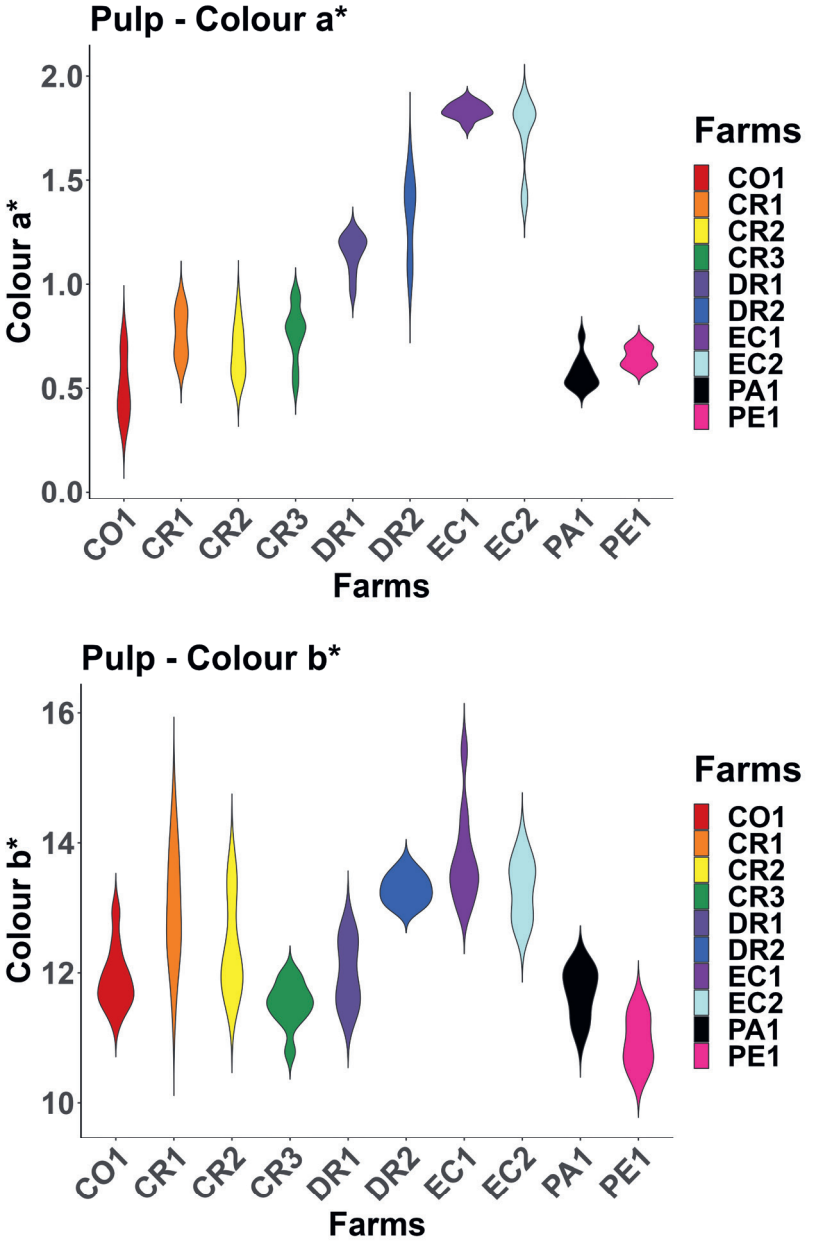
(a) Continued


Pulp - Total Dietary Fibre
Total dietary fibre (g/100 g)
24
21
18
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Farms
Farms
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Pulp - Protein
Protein (g/100g)
3.75
3.50
3.25
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Farms
Farms
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1

(a) Continued

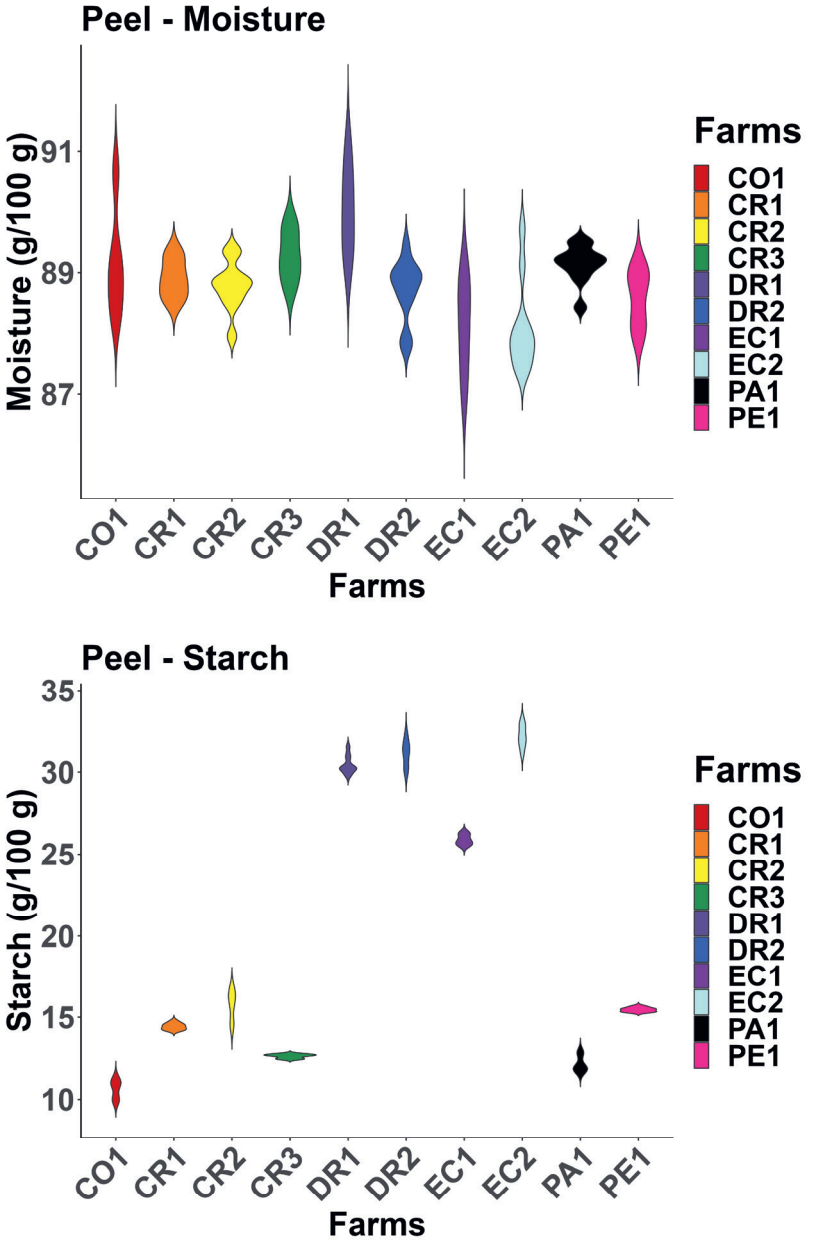

Pulp - Carotene
Carotene (µg/mg)
4
3
2
1
0
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Farm
Farms
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Pulp - Colour L*
Colour L*
87.5
85.0
82.5
80.0
77.5
75.0
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Farm
Farms
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1

(a) Continued

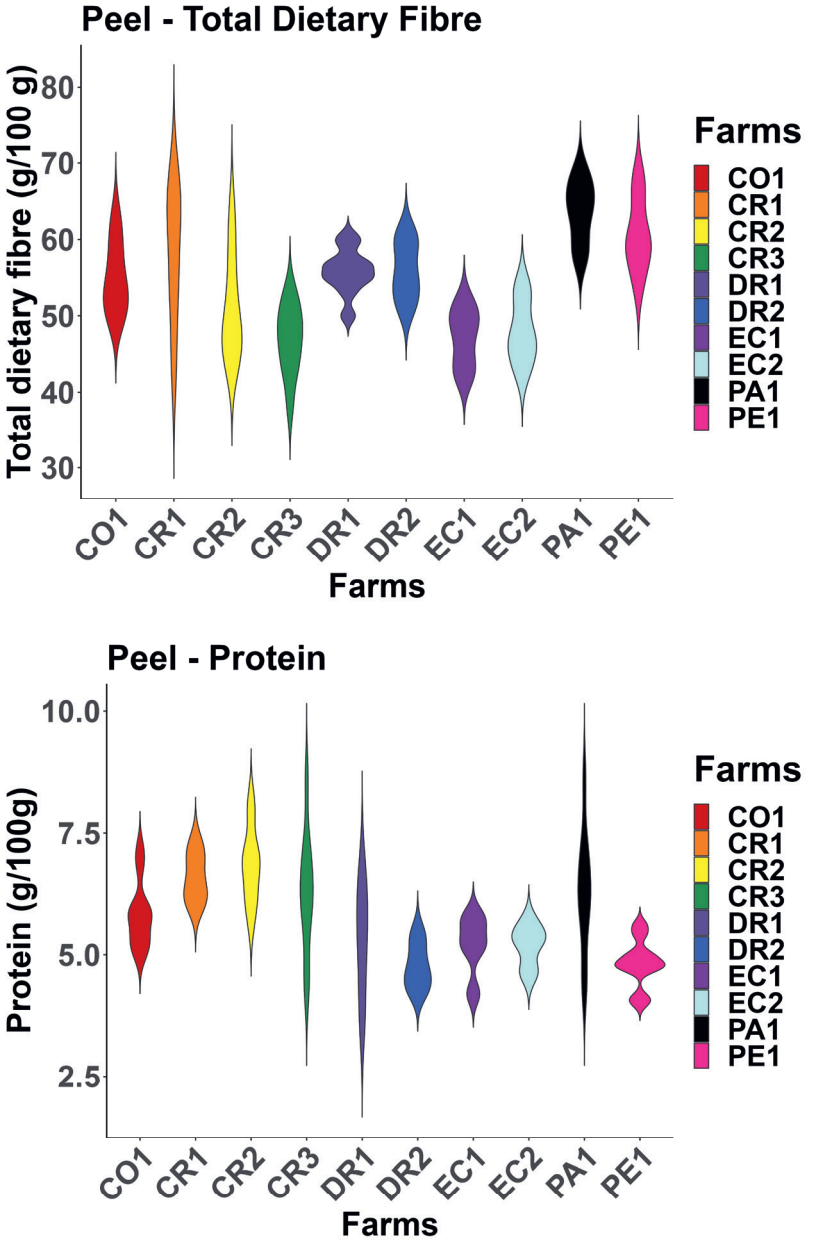


5

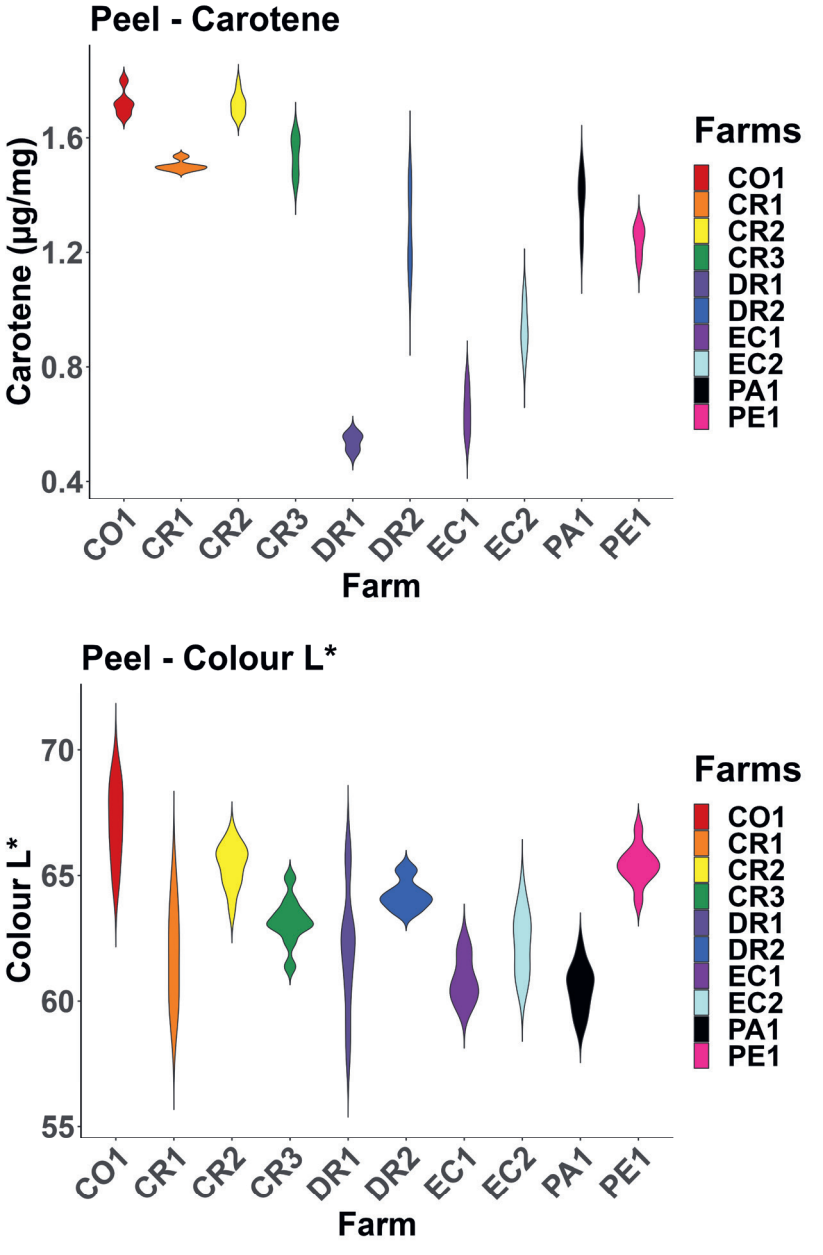
(b)


Peel - Moisture
Moisture (g/100 g)
91
89
87
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Farms
Farms
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Peel - Starch
Starch (g/100 g)
35
30
25
20
15
10
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Farms
Farms
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1

(b) Continued


Peel - Total Dietary Fibre
Total dietary fibre (g/100 g)
80
70
60
50
40
30
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Farms
Farms
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Peel - Protein
Protein (g/100g)
10.0
7.5
5.0
2.5
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Farms
Farms
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1

(b) Continued


Peel - Carotene
Carotene (µg/mg)
1.6
1.2
0.8
0.4
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Farm
Farms
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Peel - Colour L*
Colour L*
70
65
60
55
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1
Farm
Farms
CO1
CR1
CR2
CR3
DR1
DR2
EC1
EC2
PA1
PE1

(b) Continued

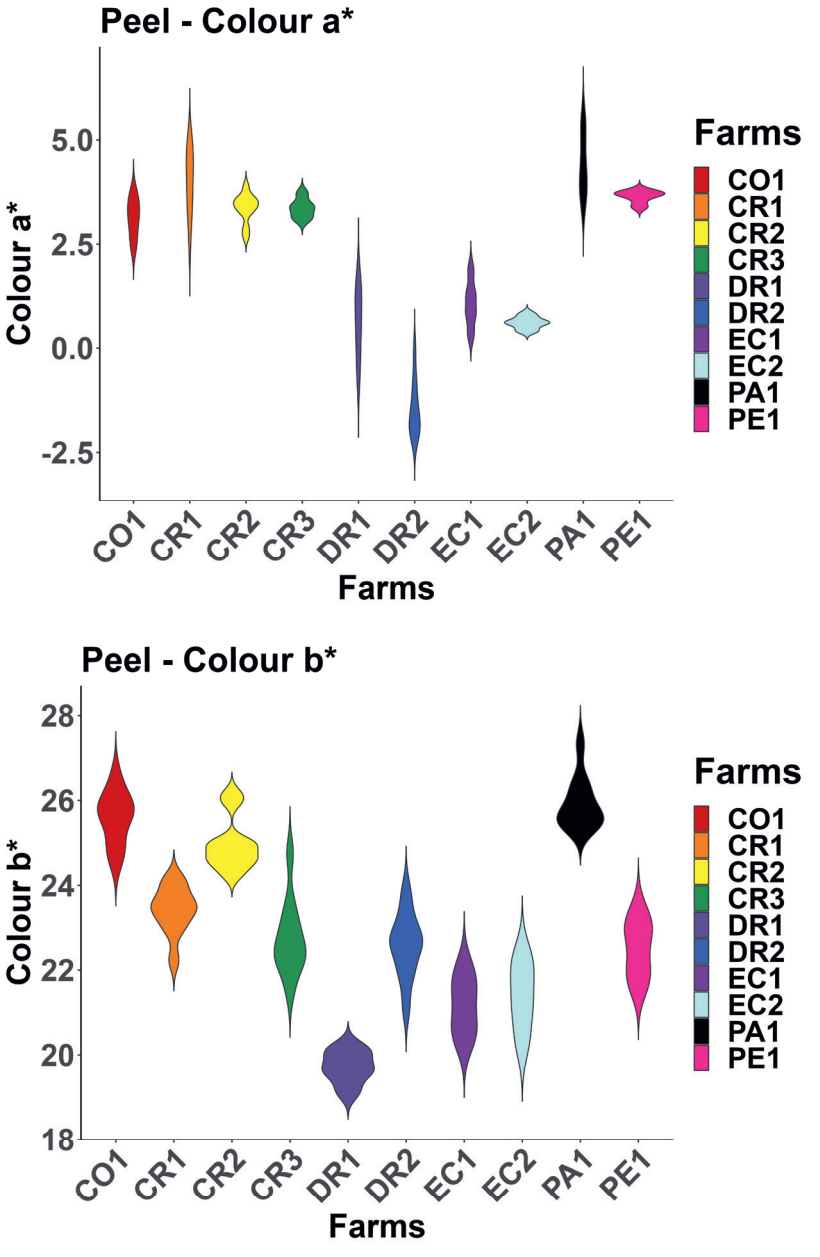


Figure A5.1. The violin plots of to show the data distributions of chemical compositions and colour values of banana pulp (**a**) and peel (**b**). (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

CHAPTER 6

6

General discussion and conclusions

6.1. Introduction

In recent years, the increasing numbers of exports and imports of bananas indicates a big demand from consumers for the fruit. Moreover, there is a particular interest for high quality bananas from certified geographical origins and/or organic production systems. The banana supply chain has gradually become a potential target for fraudsters with its continuous growth in trade volume. Mislabelling of geographical origin and production system in recent fraud incidents reflects the importance to detect food fraud in banana supply chains. Important questions are:

- How can one verify if the claims on the labels match the true quality of the fruit?
- How can one predict the compositional parameters of bananas according to their growing conditions (e.g., geographical features, production system)?
- How can one use this correlation with results to mitigate food fraud in banana and related products?

These questions are worthy of an in-depth understanding of the relationship between the compositional characteristics of bananas and their growing conditions. The main findings of this thesis are summarized in Figure 6.1.

In response to the above questions, the compositional analysis of bananas was conducted (**Chapter 5**) and intrinsic markers affected by geographical origin and production system including stable isotopic ratios, elements and volatile/non-volatile compounds were investigated, while the spectral characteristics such as Hyperspectral imaging (HSI) and colour values were also integrated to explore their relationship with growing conditions (**Chapter 2-5**). The effects of growing conditions on the banana characteristics were discussed in each chapter. Furthermore, the correlation network based on the compositional characteristics and growing conditions of bananas will be generated in this chapter to contribute to the banana origin prediction map (**Chapter 6**).

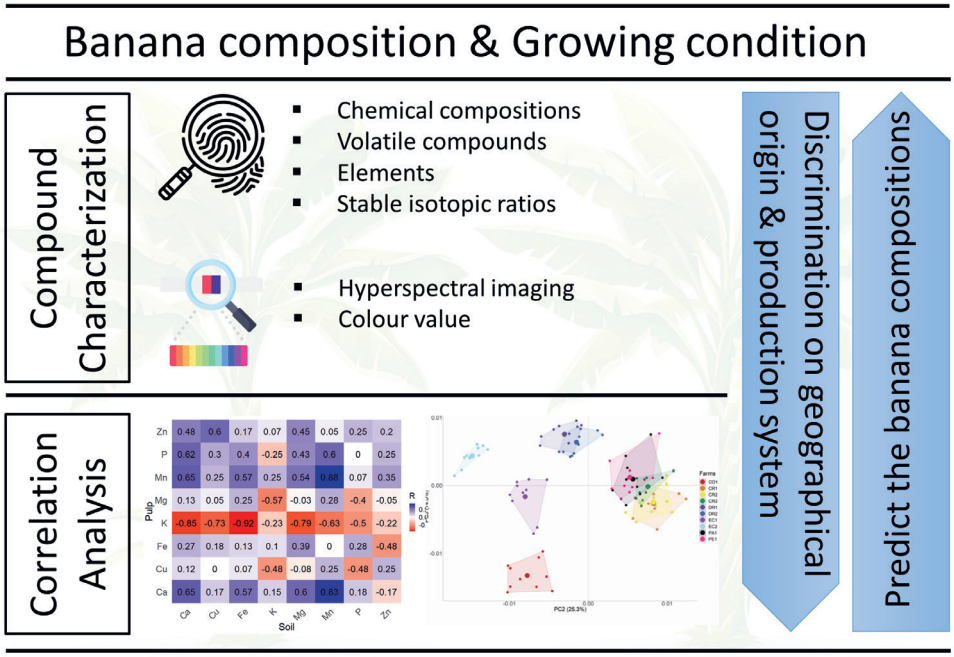


Figure 6.1. The main findings of this thesis (**Chapter 2-5**).

6.2. Explorative analysis based on the compositional differences of bananas from different growing conditions

To reflect the effects of growing conditions on banana compositions, the different characteristics of bananas in chemical compositions and spectral features were investigated in Chapter 2-5. The factors influencing the banana characteristics such as rainfall, temperature, farm altitude, fertilization, distance to sea, and soil properties were studied. In this section, the main differences of banana characteristics and effects of growing conditions will be interpreted and discussed.

6.2.1. Composition of the non-volatile compounds

The influences of geographical origins and production systems on the chemical compositions and the final quality of agricultural products has been highlighted in previous studies such as the anthocyanin composition in Russian box thorn (*Lycium ruthenicum*) and the sugar compositions in Starfruit (*Averrhoa Carambola* L.) (Ramadan et al., 2020; Wang et al., 2018). In this thesis, the chemical compositions

regarding the contents of moisture, starch, total dietary fibre (TDF), protein, and carotene of banana pulp and peel were measured to explore the effects of different growing conditions (Chapter 5). The samples collected at country level showed that the qualitative differences in chemical profiles were not always significantly different among all sampling sites (the 10 farms in six countries) (Table 5.2). However, the principal component analysis (PCA) biplots based on the chemical profiles of the pulp and peel show that the samples can be more distinctly grouped based on geographical origin as opposed to production system (Figure 6.2).

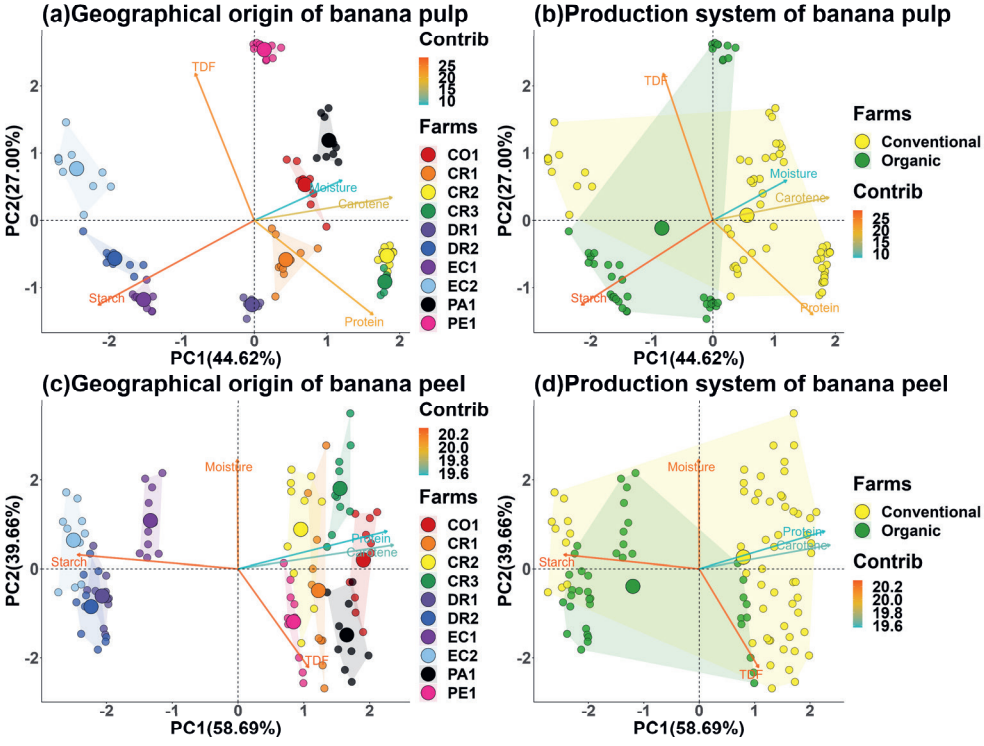


Figure 6.2. The principal component analysis (PCA) biplot of banana pulp (n = 12) and peel (n = 12) samples based on content of moisture, protein, carotene, total diet fibre (TDF), and starch of banana pulp and peel according to different geographical origins and production systems. The different coloured polygons indicate the different clusters and the arrows show the contributions of the chemical compositions. (CO) Colombia; (CR) Costa Rica; (DR) Dominican Republic; (EC) Ecuador; (PA) Panama; (PE) Peru.

As shown in the biplot (Figure 6.2), the main variables contributing to the preliminary separation of banana pulp based on geographical origin were the contents of starch, TDF, protein, and moisture. A similar trend was also found in the peel results. Similarity for geographical origin and the effects of production system on the chemical profiles for the pulp and peel were not always different (Figure 6.2b and 6.2d). The overlapped regions (polygons) in the PCA plots among all the sampling sites could be due to organic fertilisers also being used in conventional farms for soil controls. This finding was consistent with a study about kiwi fruits' compositions, where the contents of sugars and organic acids were not affected by organically and conventionally grown systems (Amodio, Colelli, Hasey, & Kader, 2007). Similar grouping were found in the DART-HRMS results. Chapter 4 reported that the DART-HRMS results further indicated that the signal strength of 18 non-volatile compounds like zeaxanthin, sucrose, etc. were different for samples collected from different farms. The PCA plots using the non-volatile compounds showed that the production systems (e.g., organic, conventional system) could be separated preliminarily (Figure 4.5). In conclusion, the primary compositions can be linked with both the geographical origin and production system of bananas.

6.2.2. Composition of the volatile compounds

In this thesis, the volatile compounds were studied as a potential intrinsic characteristic to distinguish the geographical origin and production system of bananas (Chapter 4). As reported, the volatile compounds are closely related to the metabolism of plants, and the metabolites of plants are directly affected by the climate and planting methods of the production area (Ch et al., 2021). Therefore, the application of volatile organic compound analysis could be used to trace the geographical origin and production system. For instance, the geographical origin and primary processing step of cocoa beans are reflected by their volatile signatures (Acierno, de Jonge, & van Ruth, 2020). Liu et al. (2018) established the relationship between organic milk and cattle forage by their volatile fingerprints. In Chapter 4, overall, 120 samples from ten farms located in six countries in Latin America were analysed using HS-SPME-GC–MS. Ultimately, 18 kinds of different volatile compounds were identified. The principal component 1 (PC1) and 2 (PC2) could separate banana pulp samples according to different locations and production systems (Figure 4.3). The reason behind the separation could be the differences in growing conditions which may influence the contents of main volatile

compounds such as 3-methyl-butanol, 2-butyl-2-octenal, butanol, and 3-methyl-1-butanol-acetate. Due to the limited number of banana samples, rigorous distinguishing criteria cannot be obtained from the volatile compounds, but the finding in this section could provide new data analysis and theoretical support for the use of volatile compounds to identify the geographical origins and production systems of fruits.

6.2.3. Elements and stable isotopes

The linkages between growing conditions and plants have been widely revealed by elemental compositions and stable isotopic ratios in recent years (Camin et al., 2017). Essentially, the elements of plants are highly related with the elemental contents of the soil in which they are cultivated. The absorption, transportation, and enrichment of specific elements from soil to plant tissues are also influenced by soil pH, fertility, microbial activity, humidity, texture, etc. (Kelly, Heaton, & Hoogewerff, 2005). Similarly, the natural abundance of stable isotopes is also affected by many natural phenomena such as evaporation, condensation, absorption, desorption, diffusion, and thermo-diffusion (Carter & Chesson, 2017; West, Bowen, Dawson, & Tu, 2009). These geographical features will cause the fractionation of heavy and light isotopes in water, soil, and plants. Therefore, the stable isotopic ratios can be used to infer the geographical origin using hydrogen, oxygen, and sulphur isotope ratios and production systems by nitrogen isotope ratios (Drivelos & Georgiou, 2012; Kelly et al., 2005). In this thesis, the elemental compositions and stable isotope ratios of banana pulp and peel were characterised using samples collected from at two scales, farm level (i.e. Costa Rica) and country level (i.e., six countries in Latin America), to reflect the relations between compositions and growing conditions (Chapter 2 and 3).

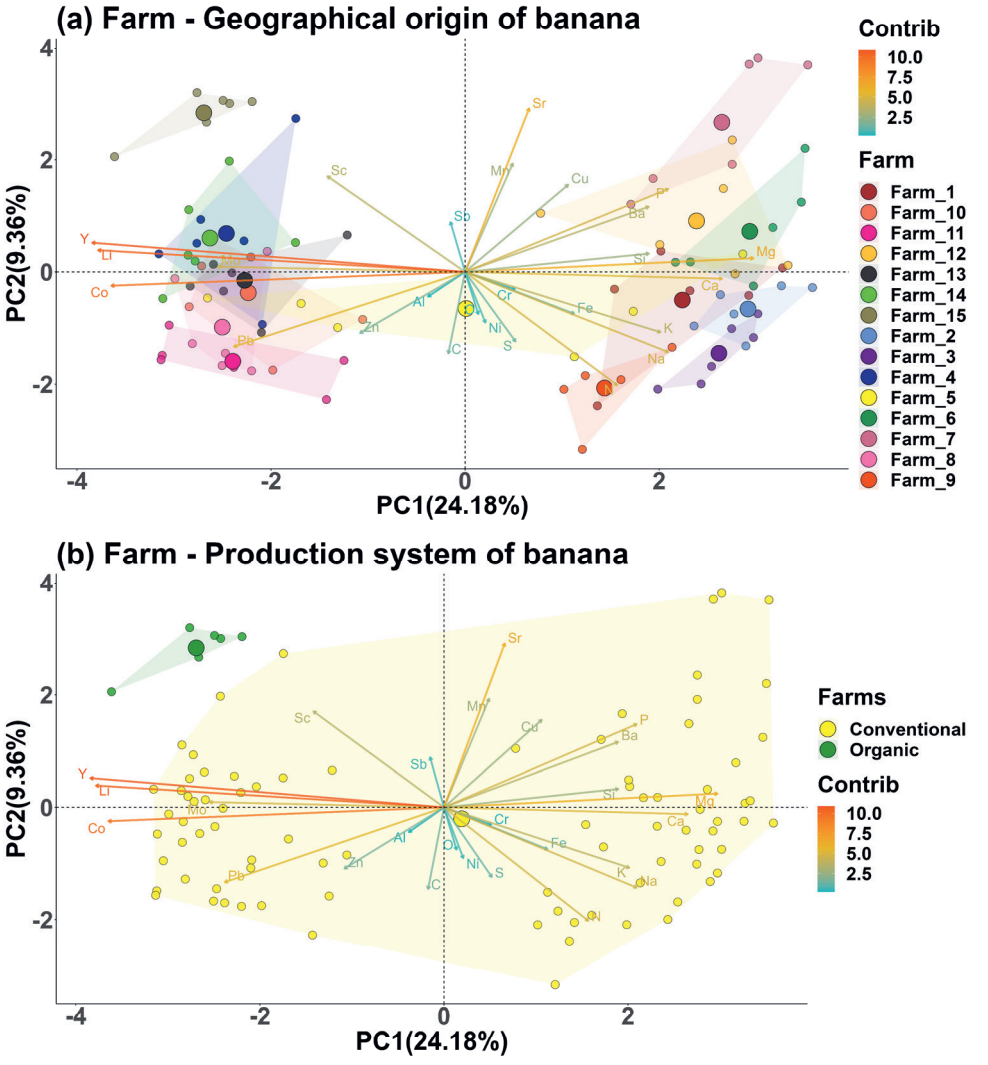


Figure 6.3. The principal component analysis (PCA) biplot of banana pulp (n = 6) samples based on stable isotopic ratios and elemental compositions according to different geographical origins and production systems. The different coloured polygons indicate the different clusters and the arrows show the contributions of the chemical compositions. (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

At farm level (Chapter 2), all the sampling sites were geographically divided into two main parts, North and South, by the *Reventazón* river (Figure 2.1). Similarly, the PCA results (Figure 2.3) showed that the elemental compositions could contribute to

the separation of northern and southern farms along the *Reventazón* river. The underlying reason could be due to the presence of volcanoes that influences the soil properties as the presence of volcanoes in the northern farms is known to provide the soil with more acidic proportions. The elements such as K, Ca, Mg, P, Mn, and Fe were the main elements found in pulp and peel samples (Chapter 2). When investigating the elements among the 14 farms in Costa Rica, the elemental compositions were indicated as the main markers to trace the geographical origin due to their contents being highly related to contents detected in the soil samples (Figure 2.4). According to Laursen et al. (2011) multi-element compositions such as Ba, Ga, and Sr were significantly different among geographical locations for wheat grains. In addition, when stable isotopic ratios and elemental compositions were combined to generate the PCA results, the biplot showed that the elemental compositions contributed more than stable isotopic ratios for geographical separations (Figure 6.3). However, Figure 6.3b showed that the effects of production systems on banana compositions were more distinct for the stable isotope $\delta^{15}N$ values than the elemental compositions. For instance, the banana samples from organic farm 14 had significantly lower $\delta^{15}N$ values when compared with the conventional farms in Costa Rica. A similar finding was also reported in a study comparing organic and conventional farming systems for European raspberries, blackberries, blueberries, currants, and strawberries, where the authors reported that the different fertiliser could influence the $\delta^{15}N$ values for most of the samples (Perini, Giongo, Grisenti, Bontempo, & Camin, 2018). In the current agricultural practice, organic fertilisers are also widely used in non-organic farms. Therefore, there are many studies reported that indicate that only using $\delta^{15}N$ values to identify farm production methods have limitations (Šturm, Kacjan Maršić, & Lojen, 2011). But at least, the isotopic differences in this thesis show that one could still distinguish the bananas from organic farms from those from conventional farms.

At country level (Chapter 3), the elements and stable isotopic ratios of bananas sampled from six countries in Latin America were also used to explore the effects of geographical origins and production systems. PCA plots have been generated in Chapter 3 to show the differences in stable isotopic ratios and elemental compositions in banana pulp and peel from different origins and production systems (Figure 3.3). Figure 3.3a and 3.3b both showed that the combined application of elements and stable isotopes could separate the pulp and peel samples according to their

geographical origins and production systems. Similar results in other plants such as tea (Liu et al., 2020) and fermented cocoa beans (Diomande et al., 2015) also reported the discriminatory potential for the combined use of stable isotope and elemental analysis. The reason for the separated results in pulp and peel could be because the elemental and stable isotopic variations were mainly influenced by the characteristics of geographical features such as rainfall, monthly mean temperature and annual rainfall and production systems such as organic and conventional systems. Therefore, the differences in geographical origin and production system were finally visualized on PCA plots (Figure 3.3). For banana pulp, rainfall could promote the enrichment of Fe, Mn, and Fe, however, the high temperature could cause the decreased level of Fe and Mn (Figure 3.4). For banana peel, the results in Chapter 3 indicted that the contents of Mn and Ni were positively associated with altitude. The geographical related influences could be suspected as comprehensive results due to soil fertility, microbial activity, the interaction between roots and minerals (Hemkemeyer, Schwalb, Heinze, Joergensen, & Wichern, 2021; Turner & Lahav, 1985; Zhao, Zhang, & Zhang, 2017). In terms of stable isotopes, the variations of stable isotopic ratios were extremely obviously in production systems. The organic farms such as DR1, DR2, and EC1 had significantly higher $\delta^{15}N$ values than other conventional farms. Therefore, the $\delta^{15}N$ values can be marked as the main drivers for the separation of geographical origins and production systems in pulp and peel samples. Regarding the contribution of elemental compositions, for example of pulp samples, the main contributions came from Mo, N, Sr, Rb, and Ni (Figure 3.3). However, the overlapped regions in the PCA results indicated that the combination of elemental compositions and stable isotopes were also facing difficulties to group geographical origins and production systems clearly. A larger number of samples and growing conditions should be considered for improving the correlation analysis.

6.2.4. Spectral characteristics

Portable devices and rapid detection are part of the recent trends in food authenticity research. Regarding the practical application, traditional devices often consume lots of expensive reagents and test time. Spectroscopy technology is a scanning method that can effectively improve test efficiency. In this thesis, the HSI spectroscopy were applied to highlight the recognized pattern of geographical origins and production systems of bananas (Chapter 5).

In principle, the difference in intensity of characteristic peaks in spectral can reflect the compositional difference between different banana samples, which can be illustrated in PCA plots (Osborne, Fearn, & Hindle, 1993). The main characteristic wavelength ranges for banana pulp in HSI were 400–500, 640–700, and 850–920 nm (Chapter 5). For geographical origin, PCA plots using HSI spectral showed that most of farms could be speared in geographical origins and production systems with minimal sample pre-treatment (Figure 5.3), because the difference in the spectrum is ultimately due to the difference in the composition of the banana. Similar advantages of HSI in identifying the geographical origins and production systems of agricultural products were also reported in spices and herbs (Oliveira, Cruz-Tirado, & Barbin, 2019). The correlation analysis in Chapter 5 indicated that the spectrum differences could be linked to compositional differences. It was found that HSI spectral reflect the difference in colour values of banana samples. These findings indicate the potential of spectroscopy in identifying the geographical origin of food, such as simple sample pre-treatment, fast speed, and accuracy. Combined with chemical compositions and correlation analysis, spectroscopy technology can be widely used to identify the geographical origin of food and infer the influence of the geographical features on the compositional differences. Ultimately, HSI can provide more characteristic information for further analysis by scanning the entire sample at high resolution (in space). The potential application of HSI as a rapid detection method for geographical origin and production system is consistent with the study in organically and conventionally farmed salmon (Xu et al., 2017) and potatoes (Rao et al., 2017).

6.3. The correlation map of bananas based on growing conditions

The correlation map of bananas means to infer the compositional characteristics of bananas based on correlation analysis. The objective is to provide insight into the relationship between the growing conditions and banana compositions.

6.3.1. Farm level

The findings at farm level were related to the effects of growing conditions on elemental compositions and stable isotopic ratios of bananas. Evidence from Chapter 2 confirmed the important role of geographical features on the elemental compositions of banana pulp and peel. For instance, the altitude of farm location had increasing

effects on the contents of elements in banana pulp such as K (r = 0.63, $p < 0.05$) but decreasing effects on elements such as Li (r = −0.57, $p < 0.05$), Mn (r = −0.58, $p < 0.05$), and Mo (r = −0.62, $p < 0.05$). For banana peel, the andic soil usually caused lower concentrations of most of the elements, but the opposite effect was found in non-andic soil (Figure 2.4). In the results of stable isotopes, the ratios of nitrogen were a critical index for production systems. The findings of this thesis were also consistent with research on different European raspberries (Perini et al., 2018) and lettuce (Mihailova, Pedentchouk, & Kelly, 2014), in which the ^{15}N stable isotope value of organic products were significantly different from conventional samples.

In addition, the correlation analysis between the elements in soil and banana was conducted to reflect the effects of growing conditions directly (Figure 6.4). For banana pulp, most of the contents for the same elements showed positive correlations such as Ca, Mn, Zn. However, the situation differed for banana peel. The contents of P in banana peel were negatively correlated with soil contents, which could be caused by the fixation of phosphorus by the soil. The active iron and aluminium in the acid soil will react with phosphate ions to form aluminium phosphate precipitation, which makes the phosphorus fixed and cannot be used quickly (Urrutia, Guardado, Erro, Mandado, & García-Mina, 2013). Therefore, the elemental compositions and stable isotopic ratios of bananas were selected to further study at country level (Chapter 3).

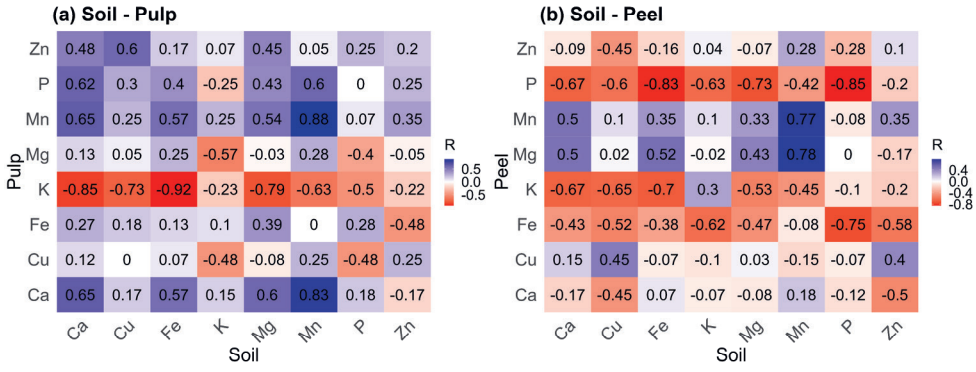


Figure 6.4. The correlation heatmap for the elements' contents between the soil and banana pulp(a) and peel (b). (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

6.3.2. Country level

In this section, a network plot was generated to show the effects of growing conditions on banana compositions (Figure 6.5). Given that the banana pulp was the main research target of this research, the heatmap was generated based on the compositional features of the banana pulp samples. Only the correlation with a coefficient greater than 0.75 was kept in the grid, which reduces overlap and mainly shows the effect of altitude, annual rainfall, monthly mean temperature, as well as organic and production systems. Overall, similar trends of correlation results between the growing conditions and banana compositions were found compared to farm level research (Section 6.3.1).

Interestingly, the effect of annual rainfall was limited at farm level in promoting the content of Zn, butylated hydroxytoluene, catechin, thiamine, carotene, and L-tryptophan. Obviously, it is difficult for rainfall to have a significant and strong correlation with most compositional characteristics. This makes sense, because irrigation is as important as rainfall for banana, and the goal is to control a reasonable amount of water in the soil. Therefore, the effects of altitude and temperature are likely stronger than rainfall, which is also reflected in the network analysis. A strong positive correlation between the monthly mean temperature and pentatonic acid ($r = 0.81$, $p < 0.05$) can be seen, which indicates that a suitable temperature could influence the volatile compounds of banana pulp. Most of the correlations of altitude were strongly correlated with the stable oxygen isotope ($\delta^{18}O$) and volatile compounds (nonanoic acid methyl ester, hexanoic acid methyl ester) ($r > 0.75$, $p < 0.05$). High altitude is conducive to the enrichment of ^{18}O in the oxygen isotope, and the role of its most volatile substances is also worth emphasizing. Based on these findings, it can be postulated that bananas from high-altitude farms may have a richer aroma.

The most significant effect of organic production is to positively promote the value of $\delta^{15}N$, which is consistent with many research reports (Rapisarda et al., 2010). The principle is that the nitrogen in synthetic fertilisers mainly comes from the air accompanied by ^{14}N, while organic fertilisers such as animal manures and residues contain more ^{15}N. There is no significant relationship between the $\delta^{15}N$ and conventional productions, which indicated $\delta^{15}N$ could be used as a marker to distinguish organic and conventional bananas (Figure 6.5).

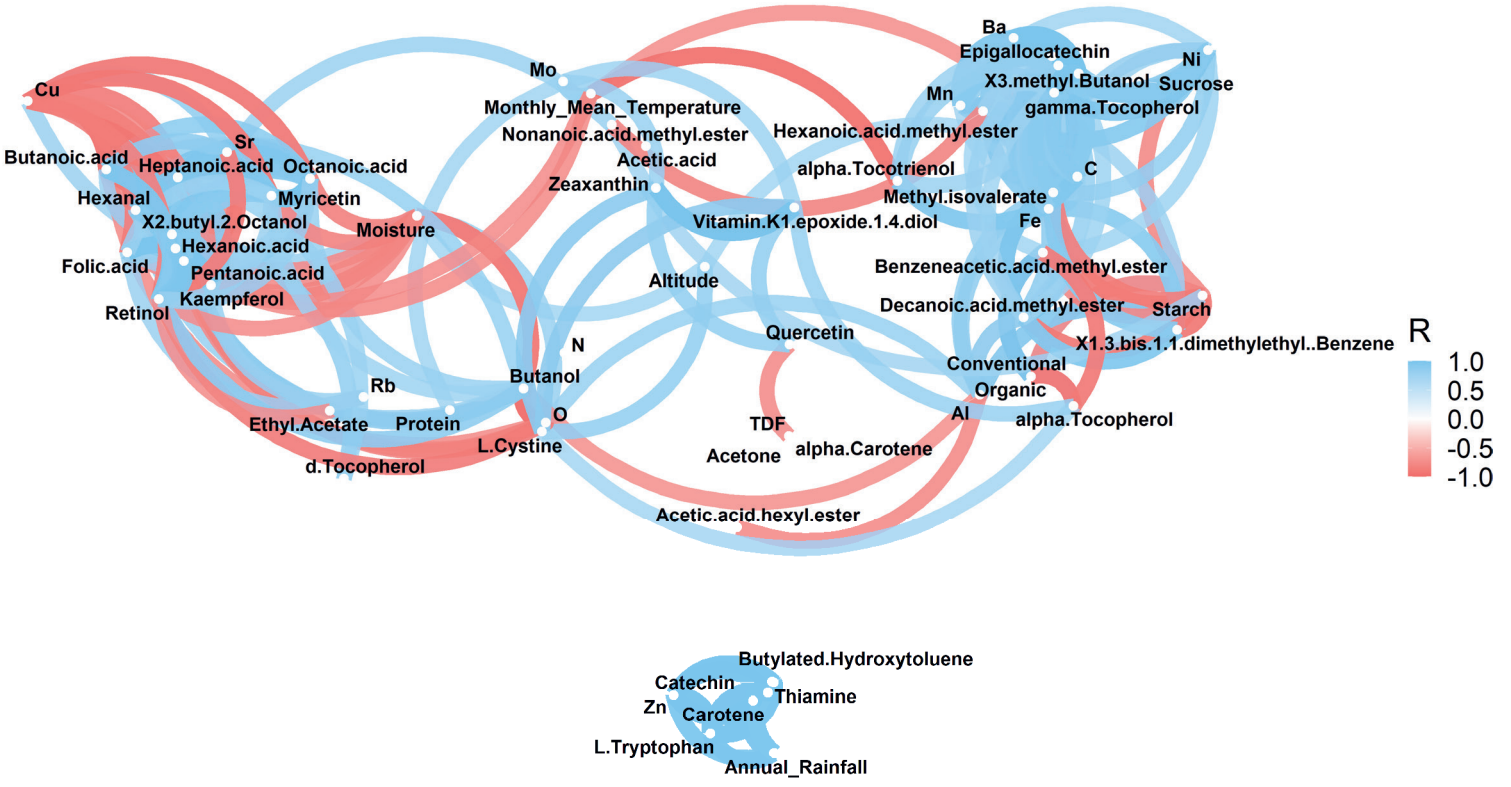


Figure 6.5. The network of banana compositions based on geographical features and production systems. (For interpretation of the references to colour in this figure legend, the reader is referred to the digital version of this thesis.)

6.4. Practical implications for banana supply chains

The findings from the correlation analysis between the banana compositions and growing conditions in this thesis firstly indicated that the compositional characteristics of bananas varied based on their geographical origins and production systems. Different compositions will inevitably lead to quality differences. As a typical imported fruit, in many consumer markets, the quality gap of bananas from different geographical origin and production systems provides opportunities for food fraud. For a long time, the research objects of food fraud are often expensive targets, such as olive oil and organic milk. It is true that the economic benefits caused by prices will indeed drive the behaviour of fraudsters. However, various news reports have shown that fraud cases that cause huge public safety incidents often occur in common but essential consumer products such as beef and infant formula. Their prices are much lower than high-valued product, such as saffron for instance, but the economic loss they cause due to food fraud is much higher than that of more expensive products. This thesis shows that although bananas are not expensive fruits in many markets, the difference in their quality and the associated commercial value will inevitably have a potential risk of fraud. The findings of this thesis can prompt the regulatory authorities and the participants in the supply chain to adopt suitable detection methods and improve regulations to deal with the risk of bulk food fraud, as represented by bananas, in advance.

Any food fraud mitigation strategy needs proper authentication methods as the first step. The findings in this thesis addressed the advantages of using spectroscopic methods like HSI. The characteristic spectrum can effectively characterize the difference in composition due to the growing conditions. The accuracy of discrimination models of origin and production system has also been confirmed by other traditional spectrometric methods. The economic cost, labour cost, and time pressure can also be released for many companies relying on the intervention of NIR spectroscopy and portable HSI cameras. For instance, the farmer could detect the quality of bananas or fruits through handheld NIR equipment, and fruit importers can also use HSI to quickly identify potential adulteration in fruits and even their processed products.

The main findings of this thesis are that the compositional features of fruits can be influenced by local growing conditions on the one hand, while the growing

conditions could also be used to predict the compositional features on the other hand. It is, therefore, suggested that the fruit quality can be predicted if sufficient and effective growing data such as geographic location, altitude, river, irrigation, rainfall, fertilization, etc. are obtained. Although our preliminary results indicated that the compositional characteristics of bananas are mainly related to factors such as rainfall, temperature, farm altitude, distances to sea, soil property, and fertilization, the detection methods and prediction models can be extended to other fruit or crop quality prediction studies. Further industrial applications can also build on the findings of this thesis.

6.5. Final conclusions

The objective of this thesis was to explore the linkage between the compositions of bananas and growing conditions. The compositional characteristics including chemical compositions, volatile compounds, elements, stable isotopic ratios and spectral characteristics obtained by HSI were compared for the banana samples obtained from different farms. The effects of geographical origin and production systems were evaluated through correlation analysis in terms of compositional and spectral characteristics.

The possibility of linking the growing conditions to banana compositional features were shown in this thesis. The geographical features influence the compositional profile of banana pulp and peel. The chemical compositions were affected by rainfall, temperature, farm altitude, distances to sea, soil properties, and fertilization. The effects of geographical features on the elemental compositions and stable isotopic ratios of bananas could happen with soil as an intermediary. The production system also influences the volatile characteristics and ^{15}N stable isotope of bananas. The use of organic fertiliser is the most important factor. However, all the differences in compositional characteristics were combined effects of geographical origin and production system, which also increase the difficulty of determining the main influencing factors.

Compared with compositional analysis, the spectral analysis with HSI allows rapid measurements. The correlation analysis also reflected the linkages between the featured wavelengths and chemical compositions of bananas.

According to the results of this thesis, the non-volatile characteristics are the most suitable for gathering detailed information on the geographical origin and production system of bananas. The application of HSI could be used to trace the geographical origin and production system of bananas as a rapid measurement. This information can complement the current traceability systems to protect the benefits of every sector in banana supply chains in advance. Overall, the rainfall, temperature, altitude, and organic fertilization were the main factors that influence banana compositions, while more geographical features were recommended to be included to generate prediction maps for banana authentication.

6.6. Research limitations and recommendations

There are some limitations in this research that could be addressed in future research. Firstly, the sampling sites of this research differed in location at country level as all the banana farms were selected within Latin America, whereas other main cultivation areas such as Southeast Asia and Africa were not included. The results of the current study can only provide a fraud mitigation strategy for banana chains from South America and the EU market. The present sampling design also resulted in limited variation in geographical features. For example, the banana farms in South Asia could have a lower altitude but higher annual temperatures. Therefore, due to the lack of sufficient geographical features, the application range of the banana prediction map is limited. In future research, the sampling farms need to be expanded to a global scope. At the same time, more samples are recommended to be included in the establishment of a characteristic banana database. Although the differences in varieties have been eliminated in this thesis, more samples in each farm can further reduce the systematic error between samples, which could improve the robustness of the prediction model.

Another limitation is related to the production systems of banana farms. Although the bananas were collected from established farms, it is impossible to control the details of farm management to characterize the effects of organic farming methods on the composition of bananas. Greenhouse research may be necessary, because the use of organic fertilisers, picking time, maturity, etc. can be strictly controlled, which is very helpful to reduce the error of the prediction model. In addition, the ripeness is also important to consider in the practical application for geographical origin and production systems of bananas. Because it will also affect certain compositional features very

much such as content of starch and volatile compounds. The comparation of effects of different ripeness for banana compositions are also important for following research.

A further limitation is related to the sample pre-treatment. To prevent the rot and weaving of bananas for long-term research, freeze-drying was the first sample preparation step before all the compounds were analysed. This could obviously affect the content of volatile substances. At the same time, powder samples make it difficult to study the differences in the three-dimensional structure of bananas caused by geographical origins and production systems. Therefore, future research on fresh bananas is highly recommended. Considering that many new portable devices are becoming available on the market, follow-up research can consider using portable devices to take images directly at the banana farm, and then transfer the data to a cloud where it is further statistically analysed. Portable laser induced breakdown spectroscopy (LIBS) for rapid elemental analysis may also be useful. In addition, in the data analysis part, a series of data science tools were applied in the data analysis process such as ANOVA, PCA and other non-supervisory data visualization methods. It is recommended to build an accurate, high-speed, open-access data analysis platform in the field of food fraud and authenticity research. High performance computing cluster (HPC) could be used for complex and supervised statistics. Just as similar analysis platforms are paired with life sciences, which can accelerate the efficiency of data analysis and can also attract the industry to share the research results of food fraud.

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Summary

Summary

Although bananas are one of the most consumed tropical fruits, the integrity of bananas in the banana supply chain have not been studied in detail (**Chapter 1**). The most notable question is mislabelling on geographical origin and production system. To ensure the integrity of bananas in the chain, extrinsic and intrinsic features can be used. This requires a thorough comprehension of the features and the underlying mechanisms involved. This thesis aimed to elucidate the correlation between the banana compositions and growing conditions, in further to predict the banana compositions according to the geographical features and production systems. Therefore, this thesis could provide the insights and analytical methods to prevent food fraud in banana chains.

To explore the effects of growing conditions on banana compositional differences, the banana samples from farm and country levels were examined in **Chapter 2 and 3** using elemental and isotope mass spectrometry. The growing conditions such as altitude of farms, distance from the sea, rainfall, and temperature were evaluated at farm and country levels. Extra soil samples including the soil type compositions and texture stratum compositions of soil were also obtained in the farm-level research. The two resolution studies showed similar correlations that most of elemental contents were associated with the altitude, temperature, and rainfall conditions of local farms. In addition, both results from farm and country level indicated that the stable isotope of nitrogen were mainly influenced by fertiliser type, however, stable isotopes of oxygen and hydrogen were more closely related to geographical features such as rainfall, temperature, and altitude. Interestingly, the acidity and alkalinity of the soil will also affect the element compositions. Hence, the geographical origin of bananas could be predicted based on the characteristics of the elemental composition.

In the previous chapters, the relationship between the chemical differences caused by growing conditions has been explained. Volatile compounds were studied in **Chapter 4** applying HS-SPME-GC-MS. As a next step, DART-HRMS was also introduced in this research chapter to analyse the compositional characteristics of bananas. There were 18 different volatile compounds were identified in the volatile fraction of banana pulp, significant differences in volatile compounds such as 3-methyl-

1-butanol-acetate, ethyl acetate, methyl propionate, and methyl isovalerate could reflect the effects of geographical origins and production systems to some extent. 18 non-volatile compounds, such as folic acid, zeaxanthin, γ-tocopherol, and L-tryptophan, were also identified from DART-HRMS analysis, and the PCA plots of non-volatile compounds could separate the samples to each production systems clearly than geographical origins. The results of this chapter further indicated that the difference in volatile and non-volatile compounds may be related to different growth conditions of bananas.

Following the profiles of the elements, isotopes and volatiles of bananas, more chemical compositions and their spectral profiles were examined in **Chapter 5**. The contents of moisture, total starch, total dietary fibre, protein, carotene content, and the colour values (CIE L*a*b*) were determined, respectively, using classical chemical analysis methods. It is worth noting that the main chemical composition does not always vary significantly with the location of the geographical origin. Significant differences are more likely to occur between farms with different geographical features, not just differences in national boundaries. This is in line with our research expectations, that is, to establish the relationship between the growing conditions and banana compositions, not just distinguish the difference between the geographical origins and production systems. To further obtain the spectral characteristics of bananas on the basis of compositional characteristics, hyperspectral imaging was performed on the banana samples. The results showed that the differences in growing conditions affected the composition of the bananas as subsequently the characteristic spectra of the bananas. The results also indicated that hyperspectral imaging is promising to identify the geographical origin and organic production of bananas rapidly.

Finally, in **Chapter 6**, a summary of the main findings, comparation of explorative analysis, implications and limitations of this thesis were provided. The most important conclusions are that the different compositional characteristics of bananas could be linked to growing conditions related to geographical origins and production systems. According to the correlation analysis, altitude, annual rainfall, monthly mean temperature, soil properties and organic fertilisers played a critical role regarding the differences in banana compositions. In addition, the spectral characteristics of bananas from hyperspectral imaging could be used for separating the geographical origins and production systems. It is recommended to further include more growing conditions and

research samples to build robust models to provide a solid foundation for fraud detection and combating the food fraud in fruit supply chains.

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If you picked up this book, you probably also are a Ph.D. candidate waiting for the light at the long tunnel. If so, then, we must know, the candidate cannot grow up to be an independent scientist alone without the help of others.

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Zhijun Wang

Overview of completed training activities

Discipline specific activities

Energy metabolism and body composition in nutrition and health research (2018), VLAG, Wageningen, the Netherlands.

IRMS – Theory and lab fruit juice and wine (2018), Food Integrity Network, York, United Kingdom.

Symposium for post-graduate students on food fraud (2018), University of Chemistry and Technology, Prague, Czech Republic.

Advanced food analysis (2019), VLAG, Wageningen, the Netherlands.

BASIS meeting (2019), BASIS, Texel, the Netherlands.

NVMS fall meeting (2019), LUMS, Leiden, the Netherlands.

Big Data Analysis in the Life Sciences, VLAG, Wageningen, the Netherlands.

33rd EFFoST International Conference 2019, EFFoST, Rotterdam, the Netherlands.

The VLAG Online Lecture Series, VLAG, Wageningen, the Netherlands.

Rheology: The do's and don'ts & Symposium, VLAG, Wageningen, the Netherlands.

China International Food Safety & Quality Conference, EU-China Project, Shanghai, China.

Chemometrics, VLAG, Wageningen, the Netherlands.

Recent advances in food analysis RAFA(2021), Prague, Czech Republic.

General courses

VLAG PhD week (2018), VLAG, Baarlo, the Netherlands.

Introduction to R (2018), VLAG, Wageningen, the Netherlands.

Applied statistics (2018), VLAG, Wageningen, the Netherlands.

Data management part 1 (2018), Wageningen Library, Wageningen, the Netherlands.

Data management part 2 (2018), Wageningen Library, Wageningen, the Netherlands.

Data management part 3 (2018), Wageningen Library, Wageningen, the Netherlands.

High impact writing (2019), WIAS, Wageningen, the Netherlands.

Adobe InDesign Essential Training (2019), Wageningen Library, Wageningen, the Netherlands.

Meta-analysis (2020), PE&RC, Wageningen, the Netherlands.

Scientific Artwork, Data visualisation and Infographics with Adobe Illustrator (2020), WGS, Wageningen, the Netherlands.

RMarkdown (2021), VLAG, Wageningen, the Netherlands.

Optional courses and activities

Preparation of research proposal (2017-2018), FQD, Wageningen, the Netherlands.

PhD study tour to Australia (2018), FQD, Australia.

Colloquia (2017-2021), FQD, Wageningen, the Netherlands.

Teaching obligation

Mentoring MSc-students and internships (2018-2020), FQD, Wageningen, the Netherlands.

FQD-36306-Food fraud and mitigation course assistant (2018-2021), FQD, Wageningen, the Netherlands.

About the author

Curriculum Vitae

Zhijun Wang was born in Shanxi, China, on the 16th of September 1991. In 2014, he obtained his bachelor's degree in Food Science and Engineering from College of Life Sciences and Food Engineering affiliated to Nanchang University in Jiangxi, China. He also finalised an Economics Bachelor in same university with project *Urban-rural dual structure and coordinated development of China*. In three years later, he obtained his master's degree in the field of *structure-function relationships of plant polysaccharides* from the same college. After that, he moved to the Netherlands where he performs a doctoral research in Wageningen University & Research since 2017, under the supervision of Prof. Dr Saskia van Ruth and Dr Sara Erasmus. His research topic is to explore the correlation between growing conditions and banana compositions. The results of this research are described in this thesis.

Contact email: zhijun0916@gmail.com

List of publications

This thesis:

Wang, Z., Erasmus, S. W., Dekker, P., Guo, B., Stoorvogel, J. J., & van Ruth, S. M. (2020). Linking growing conditions to stable isotope ratios and elemental compositions of Costa Rican bananas (*Musa* spp.). *Food Research International, 129*, 108882.

Wang, Z., Erasmus, S. W., & van Ruth, S. M. (2021). Preliminary study on tracing the origin and exploring the relations between growing conditions and isotopic and elemental fingerprints of organic and conventional Cavendish bananas (*Musa* spp.). *Foods, 10*(5), 1021.

Wang, Z., Erasmus, S. W., Liu, X., & van Ruth, S. M. (2020). Study on the relations between hyperspectral images of bananas (*Musa* spp.) from different countries, their compositional traits and growing conditions. *Sensors, 20*(20), 5793.

Wang, Z., Erasmus, S. W., & van Ruth, S. M. (2022). Exploring the effects of growing conditions on volatile and non-volatile compounds of bananas (*Musa* spp.) from different countries. In preparation.

Other work:

Wang, Z., Erasmus, S. W., & Van Ruth, S. M. (2022). Exploring the spectral differences of banana (*Musa* spp.) from different growing conditions by near-infrared (NIR) spectroscopy: A short communication. In preparation.

Wang, Z., Xie, J., Shen, M., Nie, S., & Xie, M. (2018). Sulfated modification of polysaccharides: Synthesis, characterization and bioactivities. *Trends in Food Science & Technology*, *74*, 147-157.

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