Physico-chemical quality assessment of honey from different floral and geographical origin

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Declaration of originality

I, declare that the dissertation, which I hereby submit for the degree, PhD Nutrition, at the University of Pretoria, is my own work and has not previously been submitted by me, for a degree at this or any other tertiary institution.

SIGNATURE: ______________________________
Tersia de Beer
DATE: 12 February 2024

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Abstract

Honey is a sweet, flavourful, viscous liquid food made by honey making bees from nectar or secretion of living parts of plants. In addition, it is a complex mixture with very great variations in composition and characteristics due to its botanical and geographical origin, bee species, climate and season or the nectar utilised by bees. Honey plays an important role in the livelihood of communities. The quality of honey is often neglected as demand for consumption increases. South Africa is a net importer of honey as it can only produce 50% of its demand.

Due to the ever-increasing demand for honey the risk of fraud is increasing, affecting the livelihood of communities negatively. From a legislative point of view, the South African government is currently concerned about labelling, which might be false, misleading or deceptive. Hence, food authenticity and traceability have become important issues in food testing. The composition of honey, a complex natural product, challenges analytical methods attempting to determine its authenticity particularly in the face of sophisticated adulteration. The first objective of the study was to determine if local and imported honey comply with current legislation as prescribed in the (Agricultural Product Standards (APS) Act, 1990 (Act No 119 of 1990), Regulation No R. 835 of 25 August 2000). The second objective of the study was to determine if the geographical and botanical authenticity of honey as well as possible adulteration can be accurately determined by analytical methods.

For the first objective compliance towards the Agricultural Product Standards (APS) Act, 1990 (Act No 119 of 1990), Regulation No R. 835 of 25 August 2000 was used to evaluate the quality of honey on the South African market. Data of samples from 1998 to 2017 was used. A high compliance rate of

local (90%) and imported (92%) honey samples in accordance with the APS Act, was found. Amongst the local samples, the lowest compliance was found in the Northern Cape (NC) (80%) whereas the highest was in Limpopo (LIM), North West (NW) and Free State (FS) (94%). Significant differences in physico-chemical properties were found in local honey from different floral origin with agricultural crops that differs the most from the other forage types.

The second objective of the study was to determine if the geographical and botanical authenticity of honey as well as possible adulteration can accurately be determined using the APS Act. A total of 14 honeys from different geographical and botanical origin were analysed. Honey from different geographical origin locally (n=5) and imported (n=1) and botanical origin (n=4) namely sunflower, eucalyptus, aloe and sugar cane as well as a sugar syrup were analysed to identify the sources of adulteration. Complementary tests such as pollen and stable carbon isotope analysis have been added to the set list of physico-chemical parameters to determine the geographical and botanical authenticity of honey as well as possible adulteration with adulterants. The results indicated most of the honey samples were correctly botanically identified as indicated on their labels. Four of the five *Eucalyptus* honey and four of the five *Helianthus* (Sunflower) honey were identified as unifloral *Eucalyptus* and *Helianthus* honeys respectively. In addition, both *Aloe* honeys were also identified as unifloral *Aloe* honey. The sugar syrup sample was not linked to any botanical honey type; hence it was correctly labelled. However, the pollen analysis indicated that the sugar syrup contained mostly *Eucalyptus* honey, *Eucalyptus* (E3) and sugar cane was identified as botanically not correctly named. The composition of *Eucalyptus* (E3) indicated that it is a multifloral honey with a strong *Helianthus* contribution, while the sugar cane contained mostly *Eucalyptus* honey. Both *Eucalyptus* (E4) and Sunflower (S4) are regarded as multiflora honey. Most of the honeys and syrups, with exception of the *Aloe* honeys (A1, A2) reflect a vegetation dominated by crops (*Helianthus*, *Eucalyptus*, Brassicaceae, *Citrus, Trifolium,* etc.) and weeds (e.g., Plantago). Geographical origin was not determined, although some pollen indicators reflect the floral species where the nectar has been collected.

The carbon isotope analysis alone was not sufficient to determine potential adulteration. The South African honeys with $\delta^{13}C$ values between -23.5‰ and -21.5‰ fall within a grey area, as the carbon isotope ratios obtained for these samples suggest that these products may be adulterated to a degree, whilst those with a $\delta^{13}C$ value of ≥ -21.5‰ may be deemed to be adulterated. The following honey isotope compositions fall within the grey area: Sugar Cane (-22.41‰), *Eucalyptus* (E5) (-21.76‰), *Eucalyptus* (E2) (-23.22‰), *Eucalyptus* (E3) (-23.27‰), Sunflower (S4) (-22.18‰), Sunflower (S3) (-23.43‰), Sunflower (S1) (-23.47‰) and Sunflower (S5) (-23.10‰).

Melissopalynology could confirm botanical origin, though it is not able to detect the addition of harvested pollen to the honey to disguise fake honey for the purpose of mislabelling the floral origin of the honey. In addition, geographical origin could not be confirmed neither by melissopalynology, nor by stable carbon isotope analyses. Sugar adulteration could not be confirmed by stable carbon isotope analyses. Due to the lack of data the South African regulatory tool the APS Act, were inadequate to verify authenticity and to detect sugar adulteration. Little melissopalynology information is available for southern African honey and detecting adulteration by means of added sugars with carbon isotope analysis is currently limited because only bulk honey samples are analysed without protein extraction. There is no single test which can reveal all about a honey sample in terms of adulteration and authenticity. Methods have strengths and weaknesses and it is thus advisable to combine methods complementing each other as part of authentication of honey.

Food safety monitoring is the mechanism which is used to evaluate on a continuous basis the food safety hazards, the correct implementation of procedures and food safety compliance. This type of monitoring is deeply embedded in food safety legislation around the world. Food law(s) provide the legal authority and an adequate legal framework for the food-control activities and are managed most effectively in two parts: a basic food act and food regulations.

Food legislation always lagged behind innovation and product development, as well as the composition and proposed marketing of many functional foods, for example honey. The expansion of world economy, liberalisation of food trade, developments in food science and technology, growing consumer demand, and improvements in transport and communication and international trade in food contributes to food safety regulations and laws not to be absolute. In order, to support the food safety status of our food production and supply, effective national food control systems are essential. Hence, the foremost responsibility of food control is to enforce the food law(s) protecting the consumer against unsafe, impure and fraudulently presented food by prohibiting the sale of food not of the nature, substance or quality demanded by the consumer.

In conclusion, South Africa’s 20-year-old honey legislation and standards are in urgent need of updating as adulterated honey could falsely be considered compliant. Food fraud is riskier than conventional food safety negligence as the adulterants is unconventional, motives are deliberate, and acts intentionally, designed to avoid detection. Working together to recognise and protect honey sources via standards and certifications is an important step in the right direction to ensure that the honey on the South African market is authenticated.

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List of Acronyms

A. cerana	*Apis cerana*
SO_2	*Sulfur Dioxide*
TLC	*Thin Layer Chromatography*
°Brix	*Degree Brix*
a-amylase	*Alpha-amylase*
a-isophorone	*Alpha-isophorone*
A. mellifera	*Apis mellifera*
A.m. capensis	*Apis mellifera capensis*
A.m. scutellata	*Apis mellifera scutellata*
Abs284	*Absorbance at 284 Nanometer Wavelength*
Abs336	*Absorbance at 336 Nanometer Wavelength*
AOAC	*Association of Official Analytical Chemists*
Apis spp	*Apis Species*
APS Act	*Agricultural Product Standards Act (No 119 of 1990)*
β-amylase	*Beta-Amylase*
β-isophorone	*Beta-Isophorone*
CaI	*Cluster Analysis*
CAM	*Crassulacean Acid Metabolism*
CARA	*Conservation of Agricultural Resources Act, 1983 (Act No 43 of 1983)*
Carrez 1	*Potassium Hexacyanoferrate (II)*
Carrez 2	*Zinc Acetate*
CIPC	*Companies and Intellectual Property Commission (CIPC) - Patents, Designs and Innovation Division.*
CODEX	*Codex Alimentarius Standard*
COVID-19	*Coronavirus Disease 2019*
CV1	*Canonical Variates*
CV2	*Canonical Variates*
CVA	*Canonical Variate Analysis*
DAFF	*Department of Agriculture Forestry and Fisheries*
DALRRD	*Department of Agriculture, Land Reform and Rural development*
DFAs	*Difructose Anhydrides*
DN	*Diastase Number*
DNA	*Deoxyribonucleic Acid*
DoH	*Department of Health*

DSC	*Differential Scanning Calorimetry*
DST	*Department of Science and Technology*
DST/NRF	*Department of Science and Technology /National Research Foundation*
DTIC	*Department of Trade, Industry and Competition*
e.g.	*Exempli Gratia*
EC	*European Country*
EMA	*Economically Motivated Adulteration*
EA-IRMS	*Elemental analyzer isotope ratio mass spectrometry*
e-nose	*Electronic Nose*
e-tongue	*Electronic Tongue*
EU	*European Union*
EU Directive	*European Union Directive*
Eucalyptus spp.	*Eucalyptus species*
F/G	*Fructose/Glucose Ratio*
FAPAS	*Food Analysis Performance Assessment Scheme*
FID	*Flame Ionisation Detectors*
FLAG	*Food Legislation Advisory Group*
FSQA	*Food Safety and Quality Assurance*
FT-IR	*Fourier Transform Infrared*
GC	*Gas Chromatography*
GC-MS	*Gas Chromatography Mass Spectroscopy*
GMO	*Genetically Modified Organisms*
HACCP	*Hazard Analysis and Critical Control Point*
H_2O	*Water*
H_2O_2	*Hydrogen Peroxide*
HCA	*Hierarchical Cluster Analysis*
HCl	*Hydrochloric Acid*
HFCS	*High Fructose Corn Syrup*
HFIS	*High Fructose Inulin Syrup*
HMF	*Hydroxy-methyl-furfural*
HPLC	*High Performance Liquid Chromatography*
i.e.	*Id Est*
IAEA	*International Atomic Energy Agency*
IAEA-CH-3	*Cellulose*
IAEA-CH-6	*Sucrose*

IAEA-CH-7	*Polyethylene Foil*
IHC	*International Honey Commission*
IMP	*Imported*
IN	*Invertase Number*
IR	*Infrared Spectroscopy*
IRMS	*Isotope Ratio Mass Spectrometry*
IS	*Invert Sugar*
ISO	*International Organization for Standards*
ISS	*Invert Sugar Syrup*
$KAl(SO4)_2\cdot 12H_2O$	*Aluminium Potassium Disulfate/Alumina Cream*
L/FA ratio	*Lactone/Free Acid Ratio*
LC	*Liquid Chromatography*
LC-MS	*Liquid Chromatography Mass Spectrometry*
$LC\text{-}NH_2$	*Aminopropyl Bonded Silica*
LDA	*Linear Discriminant Analysis*
MRL	*Maximum Residual Limit*
MS	*Maltose Syrup*
MS	*Mass Spectrometry*
mS^{-1}	*Milli Siemens*
$mS.cm^{-1}$	*milli Siemens per centimetre*
$NaHSO_3$	*Sodium Bisulphite Solution*
$NaHCO_3$	*Sodium Bicarbonate*
NaOH	*Sodium Hydroxide*
NAMC	*National Agricultural Marketing Council*
NEMBA	*National Environmental Management Biodiversity Act 2004 (No 10 of 2004)*
NIR	*Near Infrared*
NLA	*National Liquor Authority*
NMR	*Nuclear Magnetic Resonance*
NRCS	*Agencies National Regulatory Compulsory Specification*
NRF	*National Research Foundation*
PC	*Principle Component*
PCA	*Principle Component Analysis*
PCR	*Polymerase Chain Reaction*
PLS	*Partial Least Squares Regression*
PLS-DA	*Partial Least Squares Discriminant Analysis*

ppm	*Parts per Million*
PTFE	*Polytetrafluoroethylene*
RI	*Refractive Index*
RSA	*Republic of South Africa*
r-value	*Correlation coefficient*
SABIO	*South African Bee Industry Organisation*
SABS	*South African Bureau of Standards*
SANBI	*South African National Biodiversity Institute*
SARChI	*South African Research Chairs Initiative*
SC	*Sugar Cane*
SCIRA	*Stable Carbon Isotope Ratio Analysis*
SDS-PAGE	*Sodium Dodecyl Sulphate - Polyacrylamide Gel Electrophoresis*
SMME	*Small, Micro and Medium Enterprise*
SPS	*Sanitary and Phytosanitary*
SS	*Sugar Syrup*
TBT	*Technical Barriers to Trade*
UKSAS	*United Kingdom Soil Association standards*
USA	*United States of America*
USD	*United States Dollar*
USDA	*United States of Department of Agriculture*
USGS	*United States Geological Service*
VPDB	*Vienna Pee Dee Belemnite*
WHO	*World Health Organization*
WTO	*World Trade Organization*
α-glucosidase	*Alpha Glucosidase*
CIE	*Commission Internationale d'Eclairage*

CHAPTER 1: THE STUDY IN PERSPECTIVE

1.1 Background to the study

The food industry is suffering globally from food fraud resulting in several challenges to protect the integrity of food products from farm to consumption. The changed and emerging demands for authentic food products, consumption patterns of consumers and increasing knowledge of quality characteristics, intensify this crisis (Strayer, Everstine, & Kennedy, 2014).

Food integrity comprises food safety, authentication, quality and trust in various food products as they are produced (Goddard, Muringai, & Boaitey, 2018). Despite the emphasis on producing high quality food products food adulteration and food fraud have been noted. Globally, the most targeted food products are olive oil, milk, honey, saffron, orange juice, apple juice, grape wine, vanilla extract and fish (European Commission, 2020). The reason for deliberate tampering with the chemical composition of food products is for financial benefit (Strayer, Everstine, & Kennedy, 2014). Deliberate alterations modify the nutrient profile of a food product and can subsequently lead to a public health risk (Rychlik, et al., 2018).

Different types of adulteration exist and is accomplished when sugars are added to enhance the taste based on the consumer's preference or by mixing cheap and low-quality honey to the expensive honey to increase the production and bee feeding (Fakhlaei, et al., 2020). Adulteration plays a major role in the fluctuation of the price of honey. The South African honey trade has also fallen prey to having adulterated honey products on the market due to the high consumer demand for honey (Guelpa, Marini, du Plessis, Slabbert, & Manley, 2017).

As is the case in the rest of the world, South Africa is experiencing a declining production in honey due to a lack of plant resources together with a concurrent increase in demand for natural products of high quality, wholesomeness and good sensory properties (Melin, Rouget, Midgley, & Donaldson, 2014). Bee products such as honey, royal jelly and propolis are all affected by the declining bee population (Donkersley, et al., 2017). A global collapse of honey production will have a severe impact on food production and food security as 50%-80% of the world’s food supply depends on honeybee pollination (Corby-Harris, Snyder, Meador, & Ayotte, 2018).

The honey production journey begins with the nectar of flowers. Honey is produced without any human intervention and is of animal origin (Marghitas, et al., 2010). It is regarded as an ancient food product. Honey has been used for generations as a sweetener, in traditional medicine, and as energy source or in religious practices linking the livelihood of many diverse cultures (Sunderland, 2011; Abdu-Raheem & Worth, 2013).

In 2016, Africa accounted for 13% of global honey production. Under the world's top 20 producing countries were Ethiopia (50 000 tons), Tanzania (30 000 tons), Angola (23 300 tons) and the Central African Republic (16 200 tons) (ApiTrade Africa, 2017). In Africa, only 2% of honey is exported due to its poor quality. Ethiopia is the leading honey and bee wax producer in Africa, the tenth largest honey producer in the world (53 782 tons) and the fourth largest producer of beeswax (5790 tons) (Tadesse, Tilahun, Woyamo, Bayu, & Adimasu, 2021). In 2020, global honey exports amounted to US$2.3 billion. Hence, global exports of natural honey increased in value by 15.3% from 2019 to 2020. The three top exporting countries were New Zealand, US$328.6 million (14.4%), China, $254 million (11.1%) and Argentina, $170.2 million (7.4%) (Workman, D, 2020b). The five biggest importers were the United States, Germany, Japan, France and the United Kingdom, which bought 51% of all natural honey imported during 2020 (Workman, D, 2020a).

Food integrity is important for the authorities that are responsible for the benchmarking of food products on the market to meet the demands of consumers for safe, high quality and nutritious food. The two critical steps to assess food integrity are traceability to origin and testing the quality by means of various analytical methods (Rychlik, Kanawati, & Schmitt-Kopplin, 2017). Assessment of food integrity increases consumer trust and consumer confidence that the government is protecting their rights and health status.

1.2 The South African context

The South African Bee Industry Organisation (SABIO) is the representative body of the beekeeping and honey industry in South Africa with its mission of representing, promoting and supporting the development of the beekeeping industry to an economically viable market that is sustainable and secured (Crewe, Masehela, Human, & Pirk, 2018).

The South African beekeeping industry is relatively small in comparison to both the global and local agricultural sectors. During the period from 1980 to 2017, the number of beekeepers declined from 25 000 to approximately 2 000. Vandalism, low honey prices, and a decline in honey production in colonies as well as ageing of the beekeepers are amongst the reasons for the decline (Simba, 2019). This has had a negative effect on the South African beekeeping industry. Currently, the records of the Department of Agriculture, Forestry and Fisheries (DAFF), from now on called Department of Agriculture, Land Reform and Rural Development (DALRRD) indicate that there are 161 608 managed colonies of honeybees and 2 641 beekeepers in South Africa; 77 088 managed colonies are situated in the Western Cape (Simba, 2019).

South Africa has a very low honey production rate compared to other countries in the Southern hemisphere. Honey production in South Africa contributes only 0.11% of the world's total production

(Hendricks, 2021). This is due to a lack of bee-friendly forage, as only 10% of land is suitable for agronomy in South Africa (Crewe, Masehela, Human, & Pirk, 2018). South Africa produces only 50% of its honey requirement rate of 4 000 tons. Since 1980, a drop in local honey production occurred (NAMC Report, 2008; Agrifusion, 2017). Data from the South African Revenue Services indicates that since 2016, honey imports increased by 6% of which 85% are from China. Since 2001, the importation of honey from China increased from 20 tons to 3 577 tons by 2017, compared to the decreasing rate of 300 tons to 3 tons from New Zealand in the same period (Business Insider SA, 2018). In 2018, South Africa imported a record volume of 4 480 tons (Crewe, Masehela, Human, & Pirk, 2019). Among the importing countries, South Africa ranked 33rd, with a value of $9,598,000. This represents an increase of 21.1% between2019-2020 (Workman, D, 2020a). In the same period (2019-2020), South Africa ranked 48th among under the top 100 export countries with a value of $2,695,000 (Workman, D, 2020b).

Commercial beekeepers in South Africa are struggling economically as input costs are high and escalating. The beekeeper’s revenue is currently derived from pollination services, honey production and other bee-related products (Agrifusion, 2017). The local honey trade is not only affected by supply and demand, but also by the foreign exchange rate for imports and exports. Imported honey is 20%-50% less expensive than South African honey, which cripples the local honey market and sales. Other factors, which can influence honey trade, are pandemics e.g., the global COVID-19 Pandemic during 2019-2020. During this period, worldwide imports and exports were severely disrupted (Crewe, Masehela, Human, & Pirk, 2018; Crewe, Masehela, Human, & Pirk, 2021). In South Africa, beekeepers have been unable to attend to their normal bee farming activities such as extracting/harvesting the honey in time. Consequently, this could lead to the harvesting and sale of inferior product on the South African market. To ensure that the bee products on the market are of high quality, bee products, such as honey, are evaluated against set standards.

Like many other, South Africa has regulatory frameworks for importing products. In South Africa, requirements for the control of selling honey or mixtures thereof are administrated under the Agricultural Product Standards (APS) Act, 1990 (Act No 119 of 1990), Regulation No R. 835 of 25 August 2000 (DALRRD, 2000). These regulatory requirements protect consumers against fraud while ensuring fair competition for the industry between local and imported honey products. The APS Act, 1990 (Act No 119 of 1990), guides the routine quality control of honey by prescribing the physico-chemical parameters that must be assessed (DALRRD, 2000).

1.3 Motivation for the study

Globalisation of the feed and food supply chains has opened the door to fraudulent activity resulting in several challenges that must be overcome to protect food products of animal origin. Fraudulent activities are committed for financial gain, and the likelihood of being brought to justice is very small. According to FoodFocus, food fraud cases both nationally and internationally reached previously unheard of levels (Everstin, 2019). In July 2018, a honey fraud scheme was exposed in Kwa-Zulu Natal, South Africa, that specifically included the adulteration of honey, where a sugar syrup solution was sold as honey (Erasmus, 2018). These challenges and problems recently experienced on the global and South African honey markets support the relevance of this study (Everstin, 2019).

Food fraud occurs globally and undermines product authenticity, including origin, quality and biological characteristics. Economically motivated adulteration (EMA) of honey is currently a common practice. It is done either directly by diluting the honey with cheaper sweeteners or indirectly by feeding bees with substitute feeding during scarcity of nectar, and by supplementing with antibiotics and other chemicals that increase the level of residues and mask the origin of honey (Brodschneider & Crailsheim, 2010; Strayer, Everstine, & Kennedy, 2014). These additives and supplement feeding change the chemical composition including the sugar profiles, the nutritional value and consequently the overall quality of honey. These additives include, but are not limited to, sucrose solutions, invert sugars, HFCS or various fruit syrups (Brodschneider & Crailsheim, 2010).

Honey is a natural sweet product produced by bees (*Apis mellifera*) from flower nectar and honeydew (DALRRD, 2000; Codex Alimentarius, 2001). Any deviation in the eating habits of honeybees to produce honey, therefore, contradicts the definition that defines honey. In such cases, a difference in quality between pure honey and adulterated honey is found when honey is tested for quality.

The importance of this study is to prompt decision makers (programme managers and policymakers) to make informed decisions when evaluating the effectiveness of the regulations to protect food integrity. The regulatory mandate of the Government is to ensure traceability, verification of authenticity and quality in the complete food chain, i.e., from production and processing to the end product. Identifying the necessary interventions by generating data faster and in more detail than before regularly to improve them is imperative in a world that is becoming global and more competitive.

1.4 Objectives of the study

The aim of the research was to evaluate the quality of honey produced from different floral and geographical origins, respectively. To attain this the following objectives were formulated:

Objective 1: Evaluate the quality of honey available on the South African market

In South Africa, the market offerings of honey consist of locally produced and imported honey, and a mixture of honeys. For honey to be suitable for consumer use in South Africa, its quality must be assessed. Honey quality is assessed and measured according to the APS Act, 1990 (Act No 119 of 1990), Regulation No. R. 835 of 25 August 2000, which is in line with the International Honey Commission and Codex Alimentarius Honey Standards. Quality determination ensures the authenticity, safety and integrity of honey in the food chain.

Objective 2: Linking the biodiverse flora system to the quality of honey (Biodiversity)

With more than 30 000 flowering plants, South Africa has a diverse floristic system that supports a variety of honey types on the market. Most honey types on the South African market are from agricultural crops, forestry and indigenous forage (Masehela, 2017). Recent consumer dissatisfaction with honey quality, has forced the regulatory authorities to verify the authenticity with respect to legislative requirements, because authenticity and quality are very important factors in the honey trade. The APS Act, 1990 (Act No 119 of 1990), Regulation No. R. 835 of 25 August 2000, was used in this study to verify the authenticity of honey.

Objective 3: Investigating the use of stable isotope analysis to monitor honey made by bees of a substitute product (Adulteration)

Governments have the obligation to ensure that safe, nutritious, authenticated and sustainable food products are available on the market. Evaluating the food production chain for food integrity is, therefore, vital. The food integrity value chain requires a multi-disciplinary approach that covers all food facets ranging from production to consumption. For honey, the approach consists of assessing chemical and microbial food safety and authentication of products derived from bees. The latter was achieved using stable isotope analysis, as well as other supportive tests namely. pH-value, total acidity, moisture, refractive index, total soluble solids, ash and Lund’s reaction, sugars.

1.5 Outline of this thesis

Chapter 1: The study in perspective

This chapter provides an overview of the study consisting of an introduction, justification of this study and gives a short description on the research methodology.

Chapter 2: Literature review

A literature review of honey monitoring according to South African legislation is presented. This review discussed the compositional differences of artificial honey and genuine honey. The chapter also describes how the differences affect the authenticity of bee products.

Chapter 3: Monitoring the quality of honey: A South African case study

The APS Act, 1990 (Act No 119 of 1990), Regulation No. R. 835 of 25 August 2000 was used as tool to assess and evaluate the quality of imported and local honey on the South African market over a period of 17 years.

Chapter 4: Linking the floral eucalyptus, sunflower and aloe origins of honey to quality: South Africa as a case study

Authentication in respect to the legislative requirements by comparative testing of honey from different origins (botanical and geographical), as well as potential adulteration. Honey samples originating from plants with different photosynthetic pathways (C3, C4 and CAM) were compared and evaluated using selected physico-chemical parameters and stable carbon isotope analysis. In addition, melissopalynology was used as a legislative tool to authenticate the botanical and geographical origin of honey, as well as for classification. Honey is classified as a monofloral if the pollen count exceeds 45% e.g., eucalyptus honey. Currently, pollen count is used in South Africa to grade honey, namely choice grade or industrial grade. Choice grade honey should have a pollen count of less than 25%.

Chapter 5: Monitoring how the quality of honey on the South African market correlates with the Agriculture Product Standards Act, 1990 (Act No 119 of 1990), Regulation No. R. 835 of 25 August 2000

The role of governments is to adopt and monitor compliance with legislation including those around food. The South African food regulatory framework consists of various laws that are enforced by different departments and institutions. For the purpose of this thesis, the APS Act, 1990 (Act No 119 of 1990) will be used to evaluate honey authentication.

Chapter 6: Significance of the study, conclusions, limitations and recommendations

The thesis concludes with a section including a summary, conclusions, limitations and recommendations. The implications of these findings and recommendations to consider in the future are presented and discussed. The lessons learned and challenges faced in this study are also discussed.

The Appendices include all other relevant documentation pertaining to the research that was conducted:

Appendix 1: Ethics approval letter. Ethical clearance was granted by the University of Pretoria ethical comity at the start of this project and is shown in Appendix 1.

Appendix 2: Approval letter from the Department of Agriculture, Forestry and Fisheries to utilize the analytical data generated by the National Analytical Services for purposes of publishing.

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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Honey is defined as a natural product produced by bees from various sources (nectar of flowers, the secretions of living plants and excretions of plant-sucking insects). The APS Act provides for the classification of bees as honeybees (*Apis* genus) and stingless bees (Melliponinae order). These well-known plant-sucking insects can collect and transform by combining with specific substances of their own and deposit, dehydrate, store and leave honey in the honeycomb to ripen and mature (Pavlova, Stamatovska, Kalevska, Dimov, & Nakov, 2018).

Figure 2.1: Pictures of African Honey Bee (Apis mellifera scutellata) and Cape Honey Bee (Apis mellifera capensis), (Allsop, 2018)

The authenticity of honey is defined by the Codex Standard as a pure product that is not allowed to contain any addition of other ingredients, nor have had any particular constituents removed from it (Codex Alimentarius, 2001). Honey is a complex mixture containing approximately 200 inherent constituents (organic and inorganic), which influence its physical characteristics and contribute to its unique compositional fingerprint profile (Chin & Sowndhararajan, 2020). This compositional profile affects its physical characteristics namely viscosity, density, electrical conductivity, optical properties, hygroscopic properties, surface tension, colour and crystallisation and these in turn are determined by its sugar content which is the main component of honey (Pavlova, Stamatovska, Kalevska, Dimov, & Nakov, 2018; Machado De-Melo, Almeida-Muradian, Sancho, & Pascual-Maté, 2018).

Colour is the physical property perceived immediately by the consumer and varies between colourless to dark according to its floral origin (Seraglio, et al., 2019). The different colours of honey are portrayed in Figures 2.2 to 2.5.

Figure 2.2: Honey harvested in spring represents light, clear colours: Spring Honey, Acacia Honey, Clover Honey, (Weyn's Honingbedrijf, 2024)

Figure 2.3: Honey harvested in summer, honey harvested has a cheery yellow colour with fruity flavours: Sunflower Honey, Summer Honey, (Weyn's Honingbedrijf, 2024)

Figure 2.4: Honey harvested in autumn tends to be darker and sharper in taste: Heath Honey, (Weyn's Honingbedrijf, 2024)

Honey is classified broadly into two categories, namely honey of botanical origin or honey production. According to its botanical origin it is subsequently, categorised into blossom/nectar, honeydew/forest, monofloral (unifloral), and multifloral (polyfloral). In addition, it can also be blended. Honey production consists of a chain of production steps namely centrifugation, filtering and pasteurization. In addition, honey can be further classified as crystallised, pasteurised, raw, strained, filtered, creamed, dried and ultrasonic honey. Honey is also classified according to the harvesting process. Honey can be harvested to produce two major types: Comb and Extracted honey (Fakhlaei, et al., 2020; Sharma, Vaidya, Kaushal, & Singh, 2020). In the same manner it is classified for its intended use, namely table honey, industrial or baker's honey (Pavlova, Stamatovska, Kalevska, Dimov, & Nakov, 2018). Monofloral honey is produced from a single plant source, whereas polyfloral honey is produced from various plant sources. Monofloral and raw honey are more valuable than polyfloral and processed honey due to their inherent therapeutic properties (Veloso, Sousa, Dias, & Peres, 2018).

Honey which is a bee product like royal jelly, bee venom, propolis, wax and bee bread, can add variety to the human diet and is used in the production of pharmaceuticals and food additives (Hristov, Neov, Shumkova, & Palova, 2020). In addition, honey can be used as a predictor for biomonitoring of the environment, and it can reflect potential sources of contaminants in given areas. Honey contamination can also occur due to the processing techniques used during its production which affect its quality and consequently, market revenue (Bett, 2017).

There are an estimated 25 000 bee species in the world of which are 500 stingless bee species belonging to the genera *Melipona* and *Trigona* that are located mostly in the Latin Americas, Australia, Africa, Eastern, and Southern Asia (Abd Jalil, Kasmuri, & Hadi, 2017; Gous, Willows-Munro, Eardley, & Swanevelder, 2017). Honey made from the stingless bee *Heterotrigona itama* is preferred by customers and its market price compared to honey produced from the *Apis spp*., is USD 100/kg, which is considerably higher than the price of *Apis spp* (Se, Ibrahim, Wahab, & Ghoshal, 2018). The genus *Apis* includes nine honeybee species which are endemic to Africa, Europe and the Middle East. Africa is home to 11 *Apis mellifera* (*A. mellifera*) sub-species (Eimanifar, Brooks, Bustamante, & Ellis, 2018).

The only two *Apis* species commercially used, are *A. mellifera* and *Apis cerana* (*A. cerana*). *A. mellifera* is the most productive in producing honey (Fakhlaei, et al., 2020). The two co-existing native South African bee subspecies are *Apis mellifera scutellata* (*A.m. scutellata*) and *Apis mellifera capensis (A.m. capensis)*, also called the African/Savanna honeybee and Cape honeybee, respectively. *Apis mellifera capensis* is predominantly present in the Western and Eastern Cape provinces, whereas *A.m. scutellata* occurs naturally in the summer rainfall regions. Even though they are found in different geographic locations and have varying morphological and behavioural features, their nutritional needs are the same (Eimanifar, Brooks, Bustamante, & Ellis, 2018). There is a hybrid zone, namely the “Siegfried line”, which divides South Africa into the Cape and Africa bee region. In this zone, *A.m. capensis, A.m. scutellata*, and hybrids of the two are found (Eimanifar, Brooks, Bustamante, & Ellis, 2018; DALRRD, 2020). In Argentina, a similar hybrid zone phenomenon is observed (SanFord, 2002).

In South Africa, honeybee colonies either occur in the wild or are managed and kept by beekeepers (Gous, Willows-Munro, Eardley, & Swanevelder, 2017; Eimanifar, Brooks, Bustamante, & Ellis, 2018). Bees play an important role in the ecosystem as pollinators of plants and agricultural crops. Wild bees complement honeybees economically and serve as a reservoir (additional source) for beekeepers. Wild bees are estimated to provide €153 billion in pollination services globally. Furthermore, wild bees can be used as an alternative pollinator in pollinator-dependent crops to address the increased demand for crop pollination. Wild bees are considered better pollinators for fruit crops e.g., apples, than honeybees (Hristov, Neov, Shumkova, & Palova, 2020).

The native honeybees, *A.m. scutellata* and *A.m. capensis* are used for both honey production and pollination of plants (Eimanifar, Brooks, Bustamante, & Ellis, 2018). Current records indicate that South Africa has an estimated 161 608 managed bee colonies of which 77 088 are based in the

Western Cape and managed by 843 beekeepers (Simba, 2019). In South Africa only 10% of the beekeepers are commercial farmers (800-10 000 hives), while the rest are seen as hobbyist beekeepers (less than 100 hives) (Guelpa, Marini, du Plessis, Slabbert, & Manley, 2017). This is possibly an underestimation as not all beekeepers are registered on DALRRD databases (Agrifusion, 2017). According to the Agricultural Pests Act, 1983 (Act No 36 of 1983) – Control Measures R858 of 15 November 1983 – relating to Honeybees, it is mandatory for all beekeepers in South Africa to register with DALRRD (Hendricks, 2021).

Conserving honeybees is important as it provides numerous benefits to the environment and plays a role in sustainability. Since the beginning of the 21st century a decreasing trend was observed for both managed and non-managed animal pollinators. This declining trend of pollinators (honeybees) is a global concern and is caused by multiple factors such as climate changes, intensification of agriculture and beekeeping practices (Hristov, Neov, Shumkova, & Palova, 2020). This phenomenon occurs less frequently in South Africa, possibly due to expert apicultural practices, greater plant diversity and the resilience to various diseases. However, with the American Foulbrood outbreak in 2015 the number of honeybee colonies were reduced with 40% in the Western Cape, contradicting the viewpoint of higher resilience to honeybee diseases (Gous, Willows-Munro, Eardley, & Swanevelder, 2017). To increase and replace honeybee colonies methods such as trapping wild swarms, hive splitting, removal of problem colonies and buying colonies from other beekeepers are used with trapping wild swarms the primary method in South Africa (Pirk, Human, Crewe, & Van Engelsdorp, 2014).

The mutualistic relationship between bees and flowering plants plays an important role in the ecosystem. Without bees, pollination cannot take place and bee pollinated plants are not able to flower. South Africa has a diverse flora consisting of an estimated 30 000 plant species which are used as an agricultural source for honey production by the native honeybees, *A.m. scutellata* and *A.m. capensis* (Guelpa, Marini, du Plessis, Slabbert, & Manley, 2017). The most common nectar sources are agricultural crops (e.g., sunflower), fruits (e.g., citrus) and wildflowers and fynbos. In South Africa, approximately 50% of the honey originates from the diverse *Eucalyptus* species (Masehela, 2017). Currently, the main source used by honeybees as forage resources are *Eucalyptus s*pecies, which are under severe threat. This predominant foraging source for bees in South Africa was proclaimed an invasive species, according to the National Environmental Management Biodiversity Act, 2004 (NEMBA), (Act No 10 of 2004) and *Eucalyptus* species are being cut down by DALRRD. This could threaten the survival of bees and leads to insufficient pollination (Masehela, Poole, & Veldtman, 2020). Sustainability of honeybees is vital as a decrease in their services as pollinators can reduce harvests by 40% (Stein, et al., 2017). All stakeholders (commercial and small-scale farmers, landowners, government, beekeeping organisations and the public) have the responsibility to contribute to the sustainability of honeybees (Masehela, Poole, & Veldtman, 2020).

South Africa's diverse agricultural landscape helps to sustain the mutualistic relationship between plants and pollinators. In this relationship plants provide pollen and nectar which are needed to meet the nutritional requirements of bees. Moreover, by transferring the pollen amongst the plants, reproduction and flowering of these plants are ensured. This plays a vital role in plant biodiversity and food production (Hristov, Neov, Shumkova, & Palova, 2020). One third of all the food consumed by humans originates from animal-pollinated plants (Gous, Willows-Munro, Eardley, & Swanevelder, 2017). Honeybees can visit between 50-1 000 flowers in during a foraging trip and can make up to 14 such trips daily. Thus, a honeybee colony consisting of 25 000 foragers can pollinate approximately 250 million flowers (Bradbear, 2009). Floral choices of honeybees play an important role in the sustainability of plants and crops (Hristov, Neov, Shumkova, & Palova, 2020). In South Africa, more than 50 crops including fruits (e.g., apples and plums), vegetables (e.g., pumpkin, zucchini and watermelon), berries (e.g., strawberries and blueberries) and nuts (e.g., macadamia and almonds) are dependent on pollinators (SABIO, 2018). However, with an increasing demand for pollinating services and consumer consumption, honey demand has decreased by 40% since 1980 (Agrifusion, 2017; SABIO, 2018). This shortage of honey in South Africa is due to various reasons such as the declining honeybee population and forage resources, which makes honey an easy target for adulteration (Gous, Willows-Munro, Eardley, & Swanevelder, 2017).

The quality of honey Is related to the impurities and adulterants it contains. Adulterants are any substances that are added to honey. Adulteration can either be done directly by the addition of sugar syrups or indirectly e.g., bee-feeding or blending with lower quality honey (Fakhlaei, et al., 2020). Other known types of adulteration are mislabelling, application of heat treatment to eliminate naturally formed crystals, colouring with sulphite-ammonia caramel and pollen filtration (Bodor, et al., 2020). Over the long term, these practices have a negative impact on the market, consumers and beekeepers.

2.2 Economic value of honey

In 2021 the global honey market was valued at USD 8,17 billion, with China the top producer of honey with 472 000 metric tons. The second largest honey producer was Turkey with a production of 96 340 metric tons, followed by Iran with 77 250 metric tons and Argentina with 71 150 metric tons. The annual global production for 2021 was 1.77 million metric tons (Shahbandeh, 2023b). It is predicted that the global honey production will exceed 11,8 billion USD by 2028 (Shahbandeh, 2023b). Factors contributing to this increase are the higher demand for specific types such as monofloral honey, of honey and honey from a specific geographical origin (Carreck, 2018).

Consumer preference for healthier food also contributes to the usage and indirectly economic value of honey (Fakhlaei, et al., 2020). China was in 2022 the leading country in exporting honey with a honey value worth of 277, 67 million UDS consisting of 10,5% of the overall export market. The second largest exporter was New Zealand with a honey value worth of 266,74 million UDS followed by Argentina with 266,73 million ESD (Shahbandeh, 2023a). In contrast, the USA was in 2022 the leading importer of honey purchasing an estimated 794 million USD of honey worldwide (Shahbandeh, 2023b). In Africa, for example, Ethiopia's is the leading honey and bee wax producer in Africa, the tenth largest honey producer in the world (53 782 tons) and the fourth largest producer of beeswax (5790 tons) (Tadesse, Tilahun, Woyamo, Bayu, & Adimasu, 2021). Most countries are net importers of honey due to the low production rate and high consumer demand, a scenario that is also true for South Africa.

The South African consumer-demand of 5 000 tons exceeds the volume that bee-farmers can produce locally (Hendricks, 2021). With a consumption rate of 3 000 tons, South Africa must import an additional 2 000 tons to meet consumer demand. South Africa has shifted from a local honey producing country to an importer since the production drop in the 1980s. Honey imports into South Africa have increased from 476 tons in 2001 to 4 206 tons in 2017. Honey imported from China increased from 20 tons in 2001 to 3 577 tons in 2017, representing a market share of 85% (Business Insider SA, 2018). In 2018 South Africa imported 4 480 tons of honey (Crewe, Masehela, Human, & Pirk, 2019). Imported honey on the South African market is 20%-50% less expensive than local honey. According to the South African National Biodiversity Institute (SANBI) the local honey industry sees an average turnover of over R3 billion (Hendricks, 2021). Honey has a low production cost with high returns, therefore, creating livelihood opportunities, especially in the urban areas (Güngör & Sen, 2018). In addition, honey beekeepers increase their income by producing mead (an alcoholic beverage obtained by fermenting mead wort that contains 8-18% (v/v) ethanol) (Ramalhosa, Gomes, Pereira, Dias, & Estevinho, 2011).

There is a constantly increasing consumer demand for natural and healthy food products. Bee products (wax, pollen, royal jelly and propolis) have grown in popularity over time. Hence, these bee products stimulate the market and are traded worldwide (Güngör & Sen, 2018). Factors affecting the honey trade are insufficient production, disorganised marketing and increasing consumer demands (Güngör & Sen, 2018). Other factors which increase the inability to address the demands of the consumer, include economically-motivated adulteration, more use of antibiotics, as well as environmental conditions. Climate change threaten honeybees by destroying its habitat and food sources resulting in a declining production rate. Consumers are increasingly interested in the origin, quality and authenticity of the honey they purchase, as honey is widely regarded as a premium product (Al-Farsi, et al., 2018).

2.3 Classification of honey

Honey is classified mainly by its botanical origin, but packaging and processing methods, regions of production and colour, are also used. Table 2.1 presents a summarised version of the categorisation of honey. Regional honey is also identified (for example, honey sold or produced in the EU), while in South Africa honey is also graded as choice and industrial grade (DALRRD, 2000; European Commission, 2002).

Honey is classified according to its botanical origin either as nectar honey (blossom) or honeydew. The EU regulation also includes a classification for mixed/blended honey (European Commission, 2002). Nectar honey, produced from the nectar of flowers, is further classified as monofloral or polyfloral. Honeydew honey is produced from the secretion of all plant parts other than flowers or excretions of sap-sucking insects on the plants. Mixed/blended honey is a combination of floral and/or honeydew honey. Blended honey can be made from two or more honeys that differ in their floral sources, colour, flavour, density or geographic origin (Eshete, 2019a). For honey to be classified as monofloral, it must have specific sensory, physico-chemical and microscopic characteristics that indicate that it was produced from a single plant (it should contain more than 45% of a single pollen originating from this plant). However, there are exceptions, if honey is produced from chestnut or eucalyptus, the pollen percentage can vary between 70%-90%, while if produced from lavender, only 15% of pollen grain is required for the honey to be declared as monofloral. Monofloral honey is of high commercial value due to its distinctive flavour, taste and biological properties (Soares, Amaral, Oliveira, & Mafra, 2017).

Polyfloral honey is produced from a variety of flora and is the predominant type sold on the market. Honeydew honey, also referred to as forest honey, is made from the sweet juices of other plant parts [of plants], excluding flowers (excretions or secretions of plant sucking insects) mixed with forest flora (Fakhlaei, et al., 2020). The interest in honeydew honey has increased lately due to its nutritional, sensorial and therapeutic characteristics (Seraglio, et al., 2019). Because information about the botanical and geographical origin of honey is a guarantee of its quality, the ability to prove its origin is crucial for obtaining and maintaining market niches. Melissopalynology (the study of pollen contained in honey and, in particular, the pollen’s source), is used to authenticate the botanical origin and region of production. However, legislation allows the removal of pollen through filtration which makes it difficult to determine the botanical and geographical origin. Honey from China masks the botanical and geographical origin by using ultra-filtration (Eshete, 2019a). Moreover, for the pollen analysis to be applied correctly, there should be distinctive differences between the geographical and climatic conditions (Berriel & Perdomo, 2019).

Table 2.1: Categorisation of honey according to floral sources and processing techniques, colour, consistency, smell and taste, style of benchmarking according to grading, (Adapted from (Sharma, Vaidya, Kaushal, & Singh, 2020)

Botanical origin–- (vegetation or forest)	Description
Monofloral/unifloral honey	Mainly from the nectar of one type of flower. Monofloral honeys have distinctive flavours and colours due their different nectar sources.
Polyfloral/multifloral honey	The nectar is derived from more than one type of flower. The type of flower affects the taste, aroma and flavour.
Honeydew honey	The sweet secretions of plant sap-sucking insects or aphids. Honeydew honey has the rich flavour of stewed fruit or fig jam, furthermore it is not as sweet as nectar/blossom honey and is dark brown.
Blended	This is a mixture of two or more honeys from different floral sources, flavours, colours, density and geographical origins. Most commercial honeys which are available on the market are blended.
Mode of processing	**Description**
Crystallised honey	Extracted honey which has crystallised/granulated to a greater or lesser extent due to the glucose content. This honey is also known as “granulated honey” or “candied honey”.
Pasteurised honey	Honey that has been heated at temperatures higher than 72 °C and then ultra-filtrated.
Raw honey	Honey as it exists in the beehive or as obtained by extraction, settling or straining, without applying heat. The honeycombs have been broken to release the honey from the cells, and this type of honey is unfiltered and unheated.
Strained honey	Has been passed through a mesh material of a particular size to remove pieces of wax, propolis and other foreign objects without removing the pollen.
Filtered honey	Honey which has been filtered to such an extent that most, or all, fine particles, pollen grains, air bubbles and foreign objects have been removed.
Creamed honey	This honey contains a large number of small crystals, which prevent the formation of larger crystals than can occur in unprocessed honey.
Dried honey	The moisture is removed to produce completely solid, non-sticky granules.
Ultrasonic honey	Honey processed by ultrasonication which is a non-thermal processing method.
Colour	The colour in liquid honey varies between clear, colourless, pale yellow, amber to dark amber e.g., bright yellow (sunflower), reddish undertones (chestnut), greyish (eucalyptus) and greenish (honeydew) (Pavlova, Stamatovska, Kalevska, Dimov, & Nakov, 2018).
Texture	Fluid, thick or partially or fully granular.
Smell and taste	Sweet with a specific honey flavour, less or more pronounced.
Grades	**Baker’s honey/Industrial:** Is suitable for industrial use and as an ingredient in other foodstuffs. Choice grade

Geographical origin plays a role in the quality of honey and its unique characteristics. Honey types differ not just from country to country, but from different regions in the same country due to floral and soil composition. Often, geographic origin is associated with low prices and quality (Soares, Amaral, Oliveira, & Mafra, 2017). The European Council Directive 2001/110/EC of 20 December 2001 relating to honey, specifically requires that the country or countries of origin where the honey was harvested must be declared. In the case of blending honey, it should be declared/stated as “blend of European country (EC) honeys” or “blend of non-EC country” or “blend of EC and non-EC country” (European Commission, 2002). An example of honey fraud through laundering with regard to geographic origin, was Chinese honey that was re-labelled German honey and exported to the USA as German honey (He, Gkantiragas, & Glowacki, 2018).

There are a variety of honey processing methods that are used to produce a product with a texture that is attractive to consumers, which has a long shelf life and is easy to bottle (Bodor, Benedek, Kaszab, & Kovacs, 2018). The qualitative and quantitative composition properties of honey are determined by the botanical and geographical origins, seasons, production practises, physical properties, chemical composition, and sensory aspects (Sharma, Vaidya, Kaushal, & Singh, 2020). Harvesting honey when it has reached the appropriate degree of maturity is important, because it is hygroscopic and can absorb moisture from the atmosphere during the harvesting process (Kadri, Zaluski, & de Oliveira Orsi, 2017).

The type of equipment used, and steps followed in processing, however, depend upon the scale of operation. The different industries can perform the different processes in different ways depending on various factors, including economic, technical and technological reasons. The processing of honey involves preheating to 40°C, straining (to remove big suspended particles), filtering/clarification (to remove fine particles), and indirect heating of filtered honey at 60°C-65°C for 25-30 minutes to reduce moisture and yeast count followed by rapid cooling in order to protect its nutritional value and quality (Eshete & Eshetie, 2019b). Hence, the mode of processing of honey is used as part of honey classification. The botanical and geographical origins can also be influenced during straining, as well as ultrafiltration. For example, strained honey or filtered honey as prescribed by legislation should be passed through a certain size of mesh (35 mesh) material to remove pieces of wax, propolis, and other defects and to prevent the removal of pollen (DALRRD, 2000; Sharma, Vaidya, Kaushal, & Singh, 2020). However, during ultrafiltration, the pollen is also removed (Eshete & Eshetie, 2019b). Thermal pasteurisation is the process mostly used in the food industry to ensure safe food. Honey is pasteurised, for a few seconds at 70ºC-78ºC and then rapidly cooled down to reduce the heat damage that affects the quality of honey, as well as to delay fermentation and granulation (Eshete & Eshetie, 2019b). During processing such as filtration, centrifugation and decantation, honey is often adulterated. For example, the addition of commercial sugars, glucose, molasses and inverted sugar solution, is commonly used as adulterants lowering the quality thereof (Salazar, Freitas, de Luz, & da Bersch, 2017).

The extracted honey should be very light and clear and should not crystallise over a long period. Comb of honey in fluid consists of pieces of comb immersed in extracted liquid honey. Creamed honey is partially crystallised, i.e., a mixture of liquid honey and crystallised honey. It is produced by controlled crystallisation and then stirred to produce a honey of uniform, soft consistency (Gopal, Joshna, & Kavitha, 2017). According to global legislation, honey is a natural substance without and shall not have any food ingredient added to it e.g., food additives or other non-honey substances (Codex Alimentarius, 2001; Soares, Amaral, Oliveira, & Mafra, 2017). In general, consumers prefer liquid honey. Hence, there is a high demand for liquid honey in markets throughout the world. It is evident that the technology used for the extraction of honey plays an important role in its quality. Some customers prefer crystallised honey (Amariei, Norocel, & Agripina Scripcă, 2020).

Honey crystallisation is a natural process, depending on the sugar content, the temperature, the water content, the storage time and botanical origin (Kadri, Zaluski, & de Oliveira Orsi, 2017). Crystallisation is an indicator that honey is pure and natural and does not adversely affect the quality of honey. However, there are types of honey that crystallise faster than others, due to their high glucose contents, such as honey produced from rape and sunflower. Other factors also contribute to the crystallisation process, such as the presence of pollen, pieces of beeswax or previously crystallised honey. Honey with a high fructose content, such as honey produced from acacia, tends to take longer to crystallise resulting in a semi-crystallised honey (Pavlova, Stamatovska, Kalevska, Dimov, & Nakov, 2018). Although there is a difference in the texture between liquid and crystallised honey, there is no difference in the flavour. The liquid phase is richer in fructose, and the crystallised phase has a higher glucose content. Methods used for preventing crystallisation, include heat treatment at high or very low temperatures, ultrasound treatment, microwave heating, filtration, ultrafiltration and addition of one or more food additives (Amariei, Norocel, & Agripina Scripcă, 2020).

In South Africa, no official standard for organic honey has been published (DALRRD, 2000). The criterion that is used to evaluate organic honey refers to the use of specified beekeeping procedures e.g., using an ecologically based system and natural sources, and not the quality. To be classified as organic honey should be free from heavy metals, radioactive isotopes, genetically modified organisms, pesticides, antibiotics and organic pollutants (Soares, Amaral, Oliveira, & Mafra, 2017). Raw honey is unprocessed honey extracted from the comb without adding heat (Sharma, Vaidya, Kaushal, & Singh, 2020).

The composition, colour, aroma, and flavour of honey are related to its botanical origin, climatic factors during nectar flow, geographical regions, manipulation, processing, packaging and storage time (Kadri, Zaluski, & de Oliveira Orsi, 2017). The colour of honey is not only a criterion for acceptance and preference by consumers, but it is also important in the international market. During crystallisation, the colour of honey changes, the product becomes opaque, with a waxy appearance, differing greatly from the initial honey sample. The colour of the original sample is determined by the floral nectar from which it is made. This colour change makes it impossible to identify a type of honey according to its original colour, that is determined by the source of the nectar (Amariei, Norocel, & Agripina Scripcă, 2020). Examples of different colours are presented in Figure 2.5.

Figure 2.5: Botanical origin attributes to the different colours of honey, (Zioga, E, 2020)

The botanical origin of honey plays a role in the texture as well. Monofloral honey, with its unique flavour and taste, is regarded as a high-quality product (Chin & Sowndhararajan, 2020). Sweetness depends mainly on the fructose and glucose content. In addition, the composition of the volatile fraction plays a role in the aroma which is influenced by the botanical origin and nectar composition (Gopal, Joshna, & Kavitha, 2017).

Grading is done to determine the quality of honey, and is based on the following characteristic moisture, colour, freedom from foreign matter, flavour and how much it has been filtered. If honey does not meet the full set of criteria for honey due to overheating, it is classified as baker's or industrial honey. Baker's/Industrial honey differs from honey as it may have a foreign taste/odour, is fermented and can be overheated. This makes it suitable to be used in industrial processes and as an ingredient in food products (European Commission, 2002). However, certain types of honey have a naturally high hydroxy-methyl-furfural (HMF) content, due to their country of origin such as Sumer honey from Oman. This high HMF value may be attributed to the summer harvesting time, where temperatures can reach up to 50°C (Al-Farsi, et al., 2018). In the grading section of the APS Act, baker's honey is grouped under industrial honey. According to the APS Act, only two grades are permitted for liquid, comb, chunk and creamed honey, Choice Grade and Industrial Grade. Grading of liquid and creamed honey is done by evaluating the following factors: colour and clarity, taste and impurities.

Comb honey is evaluated on its appearance, beeswax foundation, uncapped cells, and cells containing pollen and brood, and its taste. Chunk honey is evaluated according to its appearance comb or chunk or comb content, and how much liquid has been added to make up the nett mass and taste. Chunk honey and mixtures of bee products with added honey are currently graded as choice grade. In addition, the honey should be well ripened (minimum moisture content, maximum enzyme activity and sealed in cells with beeswax) and free from particles that are not part of its composition, not be fermented, free from foreign bodies and should not be treated in such a way that the natural enzymes are destroyed (DALRRD, 2000).

2.4 Nutritional composition of honey

Honey contains a range of macro-and micronutrients and a variety of minor substances, such as pollen grains. It consists of (80%±2%) carbohydrates, (16%±1%) water, 0.2% ash, <1% amino acids and smaller quantities of vitamins, enzymes, phenolic compounds, organic acids and other trace elements. These vary depending on the botanical origin of the honey (Fakhlaei, et al., 2020).

2.4.1 Carbohydrates/Sugar metabolism

Sugars found in honey are mostly fructose (33%-43%), glucose (25%-35%) and a small quantity of sucrose (2%) (Chen, et al., 2019). Other sugars that occur in lower concentrations are disaccharides, trisaccharides and oligosaccharides. See Figure 2.6. To date, 11 disaccharides and five trisaccharides have been identified, as well as tetrasaccharides (Se, Wahab, Yaacob, & Ghoshal, 2019). Floral honey has a high concentration of fructose and glucose, while rapeseed honey is made of more glucose, than fructose concentration (Pavlova, Stamatovska, Kalevska, Dimov, & Nakov, 2018). The average ratio of fructose to glucose is 1.2:1 in floral honey with fructose given honey its sweet taste (Aljohar, et al., 2018; Chin & Sowndhararajan, 2020). The ensymes, diastase and invertase are responsible for breaking down sugars to fructose and glucose during the storage and ripening process of honey (Silva, Sousa, & Taveira, 2017).

The sum of fructose and glucose in honeydew honey ranges between 59.9%-79.7%. In addition, honeydew honey is characterised by low levels of fructose and glucose and a higher-level of disaccharides and trisaccharides (melezitose, raffinose), which are normally not found in floral honey or are present in very low concentrations. Melezitose, is considered a marker of honeydew honey.

Due to its high fructose/glucose ratio (F/G), crystallisation does not occur often in honeydew honey (Seraglio, et al., 2019).

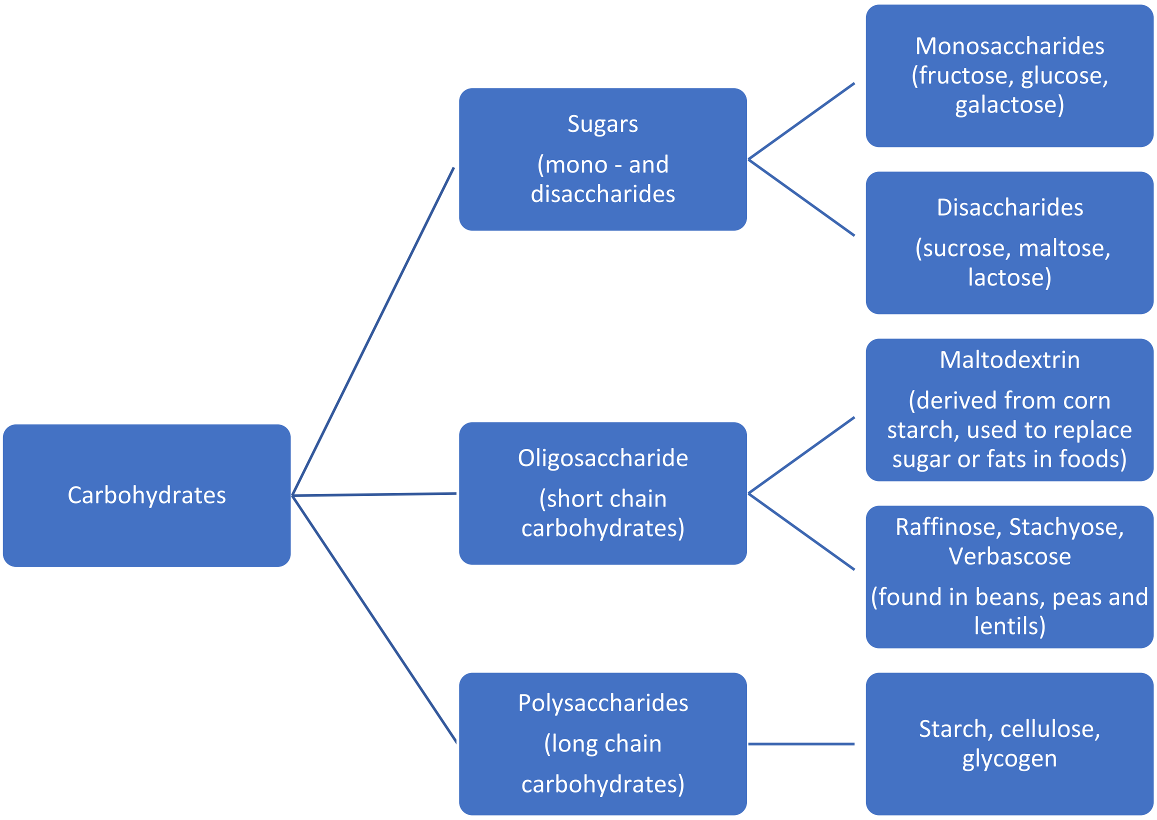

Figure 2.6: Carbohydrates can be grouped into three categories according to chemical composition and the number of single units that are bound together, (Cumming & Stephen, 2007)

2.4.2 Vitamins

Honey contains trace amounts of vitamins including B1 (Thiamine), B2 (Riboflavin), B3 (Niacin), B5 (Pantothenic acid), B6 (Pyridoxine), B8 (Biotin), B9 (Folic acid), B12 (Cobalamin), C (Ascorbic acid), A (Retinol), D (Ergocalciferol), E (Tocopherol and Tocotrienols) and K (Quinones). Vitamin C is the most abundant water-soluble vitamin found in honey. The vitamins in honey are preserved by its low pH and the vitamins content are reduced by when, it is filtered to remove pollen, as well as oxidation by hydrogen peroxide (H_2O_2) which is naturally present in honey (da Silva, Gaunche, Gonzaga, Costa, & Fett, 2016; Samarghandian, Farkhondeh, & Samini, 2017). The vitamins generally found in honeydew honey are the B group (B1, B2, B3N (Nicotinamide), B3H (Nicotinic acid), B5, and B6) and vitamin C; vitamin B1 and B2 occur in concentrations of 4.00 mg/kg and 0.16 mg/kg, respectively (Seraglio, et al., 2019).

2.4.3 Minerals

Honey contains relatively small quantities of minerals depending on its geographical (location)and botanical origin. Other factors, such as environmental pollution, climatic conditions, beekeeping practices, and honey processing techniques, e.g., extraction, influence the mineral content of the honey (Pavlova, Stamatovska, Kalevska, Dimov, & Nakov, 2018). The mineral content of honey is 0.04-0.2%, depending on whether it is light or dark and it correlates with the chemical components present in the soil, nectar and pollen (de Almeida-Muradian, et al., 2020). The dark colour of honey can be attributed to the presence of iron (Fe), copper (Cu), magnesium (Mg) and other mineral substances (Pavlova, Stamatovska, Kalevska, Dimov, & Nakov, 2018). Compared to vitamins and amino acids, mineral elements are not influenced by heat, light, oxidizing agents and pH. The mineral content of honey, therefore, plays a crucial role in its biomedical activities (da Silva, Gaunche, Gonzaga, Costa, & Fett, 2016). Table 2.2 presents the different minerals and heavy metals concentrations found in honey.

Minerals are valuable indictors to determine if honey has different floral and geographical origins. The minerals present in honey directly represent the profile of the amount of these elements present in the soil and plants where the bees collect nectar, pollen and honeydew. Both the percentage of ash and the electrical conductivity are related to the mineral content of honey (De-Melo, Almeida-Muradian, Sancho, & Pascual-Maté, 2017). Minerals are present as ash after honey has been incinerated (Jakubik, Borawska, & Socha, 2020). Previously the ash content was used to determine the botanical (floral) origin of honey. Currently, the determination of electrical conductivity is replacing the determination of ash content mainly because it is more sensitive to small changes in the mineral levels than ash content. Nectar honey has a lower ash content and electrical conductivity than honeydew honey, hence electrical conductivity can be used to differentiate between the two types of honey. Honeydew honey has a mineral content of ≤ 1.2 g/100g (w/w), and electrical conductivity > 0.8 mS^{-1} (Seraglio, et al., 2019). Moreover, electrical conductivity depends on the mineral content, ions, proteins and organic acids. The concentration of minerals in honeydew honey is often higher than 1%, with K, the most abundant followed by Ca, Mg, Na (Seraglio, et al., 2019; Jakubik, Borawska, & Socha, 2020).

The mineral content of honey is an indicator of environmental pollution (de Almeida-Muradian, et al., 2020). The minimum acceptable standards for heavy metals as set by the WHO are, honey should be free of heavy metals so as not to pose a risk to human health; however, the threshold concentration of heavy metal toxicity has not been established yet. In the interim, the World Health Organization (WHO) has proposed acceptable levels for the following: Ar (15 µg/kg); Pb (25 µg/kg); Hg (5 µg/kg) and Cadmium (Cd (7 µg/kg) (de Almeida-Muradian, et al., 2020).

Table 2.2: Minerals and heavy metals concentration in honey

Minerals	Heavy metals	Concentration range (mg/100g)	Reference
Calcium (Ca)		3 – 31	(Fakhlaei, et al., 2020)
Chlorine (Cl)		0.4 – 56	(Jakubik, Borawska, & Socha, 2020)
Magnesium (Mg)		0.7 – 13	(Fakhlaei, et al., 2020)
Sodium (Na)		1.6 – 17	(Fakhlaei, et al., 2020)
Potassium (K)		40 – 3500	(Fakhlaei, et al., 2020)
Phosphorus (P)		2 – 15	(Fakhlaei, et al., 2020)
Sulphur (S)		0.7 – 26	(Jakubik, Borawska, & Socha, 2020)
Arsenic (As),		0.01 – 0.08	(Jakubik, Borawska, & Socha, 2020)
Barium (Ba)		0.01 – 0.08	(Jakubik, Borawska, & Socha, 2020)
Boron (B)		0.05 – 0.3	(Ajibola, Chamunorwa, & Erlwanger, 2012)
Chromium (Cr)		0.01 – 0.3	(Fakhlaei, et al., 2020)
Cobalt (Co)		0.1 – 0.35	(Jakubik, Borawska, & Socha, 2020)
Copper (Cu)		0.02 – 0.6	(Fakhlaei, et al., 2020)
Fluoride (F)		0.4 – 1.34	(Jakubik, Borawska, & Socha, 2020)
Iodine (I)		10 – 100	(Jakubik, Borawska, & Socha, 2020)
Iron (Fe)		0.03 – 4	(Fakhlaei, et al., 2020)
Manganese (Mn)		0.02 – 2	(Fakhlaei, et al., 2020)
Molybdenum (Mo)		0 – 0.004	(Ajibola, Chamunorwa, & Erlwanger, 2012)
Nickel (Ni)		0 – 0.051	(Jakubik, Borawska, & Socha, 2020)
Rubidium (RB)		0.04 – 3.5	(Jakubik, Borawska, & Socha, 2020)
Silver (Ag)		0.04 – 0.35	(Jakubik, Borawska, & Socha, 2020)
Selenium (Se)		0.002 – 0.01	(Fakhlaei, et al., 2020)
Strontium (Sr)		0.04 – 0.35	(Jakubik, Borawska, & Socha, 2020)
Zinc (Zn)		0.05 – 2	(Fakhlaei, et al., 2020)
Silicium (Si)		0.0 – 24	(Jakubik, Borawska, & Socha, 2020)
Vanadium (V)		0 – 0.013	(Jakubik, Borawska, & Socha, 2020)
Zirconium (Zr)		0.05 – 0.08	(Jakubik, Borawska, & Socha, 2020)
Lithium (Li)		0.225 – 1.56	(Jakubik, Borawska, & Socha, 2020)
	Cadmium (Cd)	0 – 0.001	(Ajibola, Chamunorwa, & Erlwanger, 2012)
	Lead (Pb)	0.001 - 0.03	(Ajibola, Chamunorwa, & Erlwanger, 2012)
	Aluminium (Al)	0.01 – 2.4	(Ajibola, Chamunorwa, & Erlwanger, 2012)

The presence of the heavy metals, Pb and Cd, and toxic elements Cr and As, in honeydew honey are due to environmental contamination, mainly from soil, pharmacological treatment and processing, and storage (Seraglio, et al., 2019). It was demonstrated that honey harvested near tanneries, or iron and steel industries contain high levels of heavy metals such as Cr, Pb, As and Zn. Honey can also be used as an indicator of radioactive contamination especially due to the presence of I (Iodine), Caesium (Cs), and Ruthenium (Ru) in honey produced near nuclear facilities in Italy (Machado De-Melo, Almeida-Muradian, Sancho, & Pascual-Maté, 2018).

The mineral content of honey is also an indicator of environmental pollution caused by agrochemicals. Cadmium (Cd) contamination is a result of phosphate fertiliser usage, combustion of fossil fuels, and incineration of waste (Belas, et al., 2014). Metal contamination originates from polluted soil on which plants are cultivated. Examples of agrochemicals are organic Hg, Cd contamination as a result of phosphate fertilizers and As-based pesticides (Aljohar, et al., 2018). Other environmental pollutants include sulphate, sulphur dioxide (SO_2) and sulphonamides. The latter is especially problematic in Serbia (de Almeida-Muradian, et al., 2020). The low concentrations of Thorium (Th) and uranium (U) in honeydew honey are attributed to the low availability of these elements in nature (Seraglio, et al., 2019).

2.4.5 Enzymes

One of the characteristics that differentiates natural and artificial sweeteners and honey, is that the latter contains enzymes that originate from pollen and insects. They play an important role in converting nectar to honey and also contribute to its functional properties (Al-Farsi, et al., 2018).

Honey contains a diversity of enzymes with the main enzymes diastases (e.g., α- and ß- amylases), invertase (e.g., saccharase or α-glucosidase) and glucose oxidase, while catalase, acid phosphatase are present in lower quantities (Pavlova, Stamatovska, Kalevska, Dimov, & Nakov, 2018; Seraglio, et al., 2019; Jakubik, Borawska, & Socha, 2020; Sharma, Vaidya, Kaushal, & Singh, 2020). The various enzymes have different functions. Alpha-amylase degrades starch to a mixture of maltose, maltotriose and dextrins (di-, tri- and oligosaccharides) (da Silva, Gaunche, Gonzaga, Costa, & Fett, 2016). Sucrose is converted to fructose and glucose (invert sugars) by invertase, whilst glucose oxidase combines with water to produce hydrogen peroxide (Pavlova, Stamatovska, Kalevska, Dimov, & Nakov, 2018). The enzyme content varies due to different factors e.g., type of forage, types of honeybees, nectar collection period, quantity of the nectar flow and it sugar content. A high flow of concentrated nectar leads to a lower enzyme content and pollen consumption (da Silva, Gaunche, Gonzaga, Costa, & Fett, 2016; Alghamdi, et al., 2020). Other factors influencing enzyme content are botanical origin, storage and processing conditions as well as honey composition (Al-Farsi, et al., 2018; Seraglio, et al., 2019). Due to their high prevalence, enzymes are used as a quality indicator of honey. Diastases are quality factors for determining freshness, overheating and purity (da Silva, Gaunche, Gonzaga, Costa, & Fett, 2016; Al-Farsi, et al., 2018). Diastase activity is also an indicator of adulteration e.g., artificial feeding of bees with glucose (Guler, et al., 2014). The quality standard and freshness of honey is represented by its diastase value (DN) which is in the range of three to eight Schade units. However, the diastase value from honeydew honey is higher than 8 DN (Seraglio, et al., 2019).

2.4.6 Proteins and amino acids (Nitrogenous compounds)

Proteins in honey are derived from animal and plant origin with pollen as the main source of protein (da Silva, Gaunche, Gonzaga, Costa, & Fett, 2016). which ranges from 0-1.7% (da Silva, Gaunche, Gonzaga, Costa, & Fett, 2016). The most dominant protein in honey, is the Major Royal Jelly Protein (MRJP1) and is an indicator of adulteration (Soares, Amaral, Oliveira, & Mafra, 2017; Pavlova, Stamatovska, Kalevska, Dimov, & Nakov, 2018). The low protein levels and the difficulty in extraction and characterization by conventional methods, contribute to the lack of studies on proteins in honey (Seraglio, et al., 2019). Apalbumin 1 levels below 50 µm/g indicate adulteration with sugar syrups i.e., glucose syrup. In comparison, honeydew honey (0,6 mg/100g) contains more proteins than nectar honey (0,3 mg/100g) (Soares, Amaral, Oliveira, & Mafra, 2017; Pavlova, Stamatovska, Kalevska, Dimov, & Nakov, 2018; Dżugan, et al., 2023). Factors such as floral source, geographical conditions and temperature contribute to the variation in protein contents (Sharma, Vaidya, Kaushal, & Singh, 2020).

Honey contains 26 different amino acids (1% of total contents) which ranges between 10 and 200 mg/100g (Se, Wahab, Yaacob, & Ghoshal, 2019; Sharma, Vaidya, Kaushal, & Singh, 2020). These amino acids are derived from honeybee secretions in honey and pollen and includes aspartic acid, glutamic acid, asparagine, glutamine, phenylalanine, serine, threonine, alanine, arginine, tyrosine, valine, leucine, isoleucine, phenylalanine, tryptophan and proline (Al-Farsi, et al., 2018; Se, Wahab, Yaacob, & Ghoshal, 2019). Proline is a non-essential amino acid and the most abundant free amino acid in honey, representing 50-80% of the total amino acid content (Kivrak, Kivrak, & Karababa, 2017; Jakubik, Borawska, & Socha, 2020). In addition, it is one of the most important amino acids found in the human body, because proline is the most important component for collagen formation, it is often taken as a health supplement and dermal medication (Saito, et al., 2011). Proline is used as a criterion for honey maturity and occasionally to detect sugar adulteration (Al-Farsi, et al., 2018). The proposed quality standard for proline content in pure honey should be more than 180 mg/kg. However, proline content varies a lot and can be ascribed to botanical and/or geographical origin, processing and manipulation conditions in the profile. No specific amino acid has yet been identified as a marker for differentiating between botanical origins of honey (Seraglio, et al., 2019).

2.4.7 Antioxidants compounds

Honey contains antioxidants of botanical origin which varies with the source of nectar. Examples of antioxidants found in honey are phenolic acids, carotenoids, proteins, certain enzymes (catalase and glucose oxidase) and products caused by Maillard reactions (Nicewicz, Nicewicz, & & Pawłowska, 2021). Phenolic compounds, such as phenolic acids, flavonoids and polyphenols, commonly found in honey are considered important due to their potential role in human health (Machado De-Melo, Almeida-Muradian, Sancho, & Pascual-Maté, 2018; Halagarda, Groth, Popek, Rohn, & Pedan, 2020). Polyphenols are present in all plants and are transferred from plants to honey through the nectar collected by bees (Pavlova, Stamatovska, Kalevska, Dimov, & Nakov, 2018).

Flavonoids are sub-divided into the flavonols, flavones, flavanol, flavanones, isoflavones, anthocyanins and chalcones (Machado De-Melo, Almeida-Muradian, Sancho, & Pascual-Maté, 2018). The presence and abundance of one or more specific phenolic compounds are used as chemical markers to determine the floral origin and geographical origin of honey (Halagarda, Groth, Popek, Rohn, & Pedan, 2020). For example, quercetin, galangin, kaempferol, isorhamnetin, and luteolin are present in all types of honey. While hesperetin is unique to citrus and naringenin and luteolin characteristics of lavender (Olas, 2020). The flavanols quercetin and kaempferol are characteristic of honey derived from sunflower and rosemary, respectively (Gil, et al., 1995; Tomas-Barbera'n, Martos, Ferreres, Radovic, & Anklam, 2001). The chemical markers myricetin, tricetin, lutelin, gallic acid and abscisic acid characterise eucalyptus and protocatechuic acid for honeydew to name a few. Propolis-derived flavonoids can be used in the determination of geographical origin (Machado De-Melo, Almeida-Muradian, Sancho, & Pascual-Maté, 2018). The main functional components found in honey are flavonoids. The flavonoid content in pollen is 0.5%, 10% in propolis and approximately 6 mg/kg in honey (Pavlova, Stamatovska, Kalevska, Dimov, & Nakov, 2018).

The nature and quantity of phenolic compounds can also vary with the seasons, climatic conditions, processing and production methods (conventional or organic) and geographical origin. Hence, the antioxidant activity of the same type of honey from different geographical origins can differ (Pavlova, Stamatovska, Kalevska, Dimov, & Nakov, 2018; Halagarda, Groth, Popek, Rohn, & Pedan, 2020). The high antioxidant activity in honeydew honey is attributed to its phenolic compounds, as well as organic acids, mineral, proteins, enzymes and amino acids (Seraglio, et al., 2019).

The colour of honey, a quality criterion, is also linked to its antioxidant content. Darker honey has a stronger antioxidant activity than lighter honey (Halagarda, Groth, Popek, Rohn, & Pedan, 2020; Jakubik, Borawska, & Socha, 2020). Darker honey contains higher levels of phenolic acid derivates and fewer flavonoids (Machado De-Melo, Almeida-Muradian, Sancho, & Pascual-Maté, 2018).

2.4.8 Organic acids

Organic acids are an important group of compounds in honey and they originate from nectar or honeydew honey or from the conversion of nectar sugars (fructose, glucose and sucrose) by enzymes. In addition, some organic acids, such as levulinic and formic acids, are derived from 5-hydroxy-methyl-furfural in successive reactions (da Silva, Gaunche, Gonzaga, Costa, & Fett, 2016). Though organic acids (non-volatile and volatile acids) are only present in honey in small amounts (0.57%), they contribute to the slightly acidic nature of honey, which has a total acidity content varying between 8.68 meq/kg-59.49 meq/kg. Organic acids also contribute to honey's characteristic taste (Se, Wahab, Yaacob, & Ghoshal, 2019). Honey is deceptively acidic, as the high sugar content tends to mask the acidity of the taste. As the pH-value of honey is between 3.4-6.1, it inhibits microbial spoilage (Pavlova, Stamatovska, Kalevska, Dimov, & Nakov, 2018).

The most important and abundant organic acid in honey is gluconic acid in equilibrium with gluconolactone. Apart from the gluconic acid, other organic acids have also been identified namely: acetic, butyric, citric, citramalic, formic, lactic, malic, maleic, oxalic, pyroglutamic, tartaric, fumaric as well as succinic acids. The organic acid profiles of a honey can be used to distinguish between honey from different botanical and geographical origins. For example, citric acid is used to classify honeys according to their floral and/or geographical origins, as well as to differentiate between nectar and honeydew honey. Nectar honey contains less citric acid compared to honeydew honey (Seraglio, et al., 2019). Organic acids also contribute to the flavour, colour, shelf life, chemical properties (acidity, pH and electron conductivity), as well as the antioxidant activity the diverse types of honey (da Silva, Gaunche, Gonzaga, Costa, & Fett, 2016; Al-Farsi, et al., 2018).

2.4.9 Volatile organic compounds

Volatile organic compounds that contribute to the aroma of honey, vary in quality and quantity because of different nectar sources, microbes in honey, transformation of plant compounds by bees, honey processing and storage conditions. Volatiles in honey occur in very low concentrations and are a complicated mixture of substances with various physico-chemical properties and levels of stability (Machado, Miguel, Vilas-Boas, & Figueiredo, 2020). More than 600 volatile compounds have been identified in honey and they are used as biomarkers for identifying the floral origin of commercial honey. Therefore, the volatile compound profile can potentially be used as a fingerprint for honey authentication/quality, which can possibly be used to the identification of the origin of a specific honey (Soares, Amaral, Oliveira, & Mafra, 2017).

Only a limited number of volatile compounds are used as floral markers because the chemical composition of honey is influenced by the origin namely botanical source and location of harvesting, harvesting season, possible interactions between chemical compounds in the honey, processing and storage conditions, as well as the compound isolation and analytical procedure (Machado, Miguel, Vilas-Boas, & Figueiredo, 2020). Examples of volatiles that can be used as floral markers are: methyl anthranilate for citrus honey; cis-linalool oxide, 3-methyl-3-buten-1-ol and heptanal for acacia honey; isovaleric acid (3-methylbutyric acid), 3-methylbutanal and 2-methylbutanal for buckwheat honey; 2-aminoacetophenone, acetophenone and 1-phenylethanol for chestnut honey; α-isophorone and isophorone derivatives such as 2-hydroxyisophorone for heather honey; methyl anthranilate and lilac aldehyde isomers for orange honey and α-isophorone, β-isophorone and 4-oxoisophorone for strawberry tree honey (Soares, Amaral, Oliveira, & Mafra, 2017; Machado, Miguel, Vilas-Boas, & Figueiredo, 2020). The volatile compounds 3-hydroxy-2-butanone, acetic acid, 2,3-butanediol, 1-(2-furanyl)-ethanone, 1-hydroxy-2-propanone, trans-2-pental and 3-methylbutanol are unique to honeydew honey and could potentially be used to differentiate between the honeydew honey types (Seraglio, et al., 2019). Volatile markers for a specific monofloral honey from different areas differ due to the presence or absence of specific compounds in the flora (Machado, Miguel, Vilas-Boas, & Figueiredo, 2020).

2.5 Processing of honey by beekeepers and in the industry

The techniques for harvesting and processing of honey depend on the scale of operation in terms of economy, technique and technology, as well as consumer preferences. Commercial processing of honey includes honey purification, filtration, heating facilitating packaging and delaying granulation (Eshete & Eshetie, 2019b). Consumers prefer liquid honey, due to its visual attractiveness, which in turn increases market demand (Soares, Amaral, Oliveira, & Mafra, 2017). However, the Codex standard, EU Directive and APS Act, specify that honey shall not be treated for example heated or processed to such an extent that its essential chemical composition i.e., sugars changes or its quality is lowered (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002). Retail honey is generally subjected to some form of processing during honey production. The varies forms of processing is described below.

After harvesting, raw honey is strained and filtered to remove all impurities, such as beeswax and pollen. However, specific rules for the use of filters during filtration of honey are prescribed. According to the different legislations, filtering is allowed, but the filters used should have a mesh size not smaller than 0.2 mm to prevent the removal of pollen (DALRRD, 2000). Industrial filtration also includes fine filtration where small foreign particles, which could impede the quality of honey, are removed. When fine filtration is applied, it should be indicated as such, by including the statement “filtered honey” on the label, according to the Codex standard (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002).

Processing of honey can be done in the conventional thermal way or by pasteurisation. Heating is the most widely used method, but the optimum heating conditions rely on the botanical and geographical origins. The heating time for liquefaction, depends on the glucose concentration and on the crystal form. Thermal processing prevents spoilage and removes harmful microorganisms and reduces the moisture level to less than 20% in the honey to delay fermentation. Furthermore, thermal treatment is applied to prevent or to postpone crystallisation and to homogenise the colour of honey. This technique is limited because a high temperature tends to affect the quality and biological properties of honey. If honey is heated above 60°C the quality of honey is compromised by the presence of unstable and heat sensitive components, the decomposition of vitamins and destruction of enzymes can also happen. In addition, temperatures higher than 70°C alter the flavour, colour and granulation of honey; a breakdown of bioactive compounds and antioxidants occurs (Eshete & Eshetie, 2019b).

Thermal pasteurisation is the most widely used process in the food industry to ensure food safety. Pasteurisation consists of flash-heating for a few seconds at 70°C-78°C followed by rapid cooling to prevent heat damage. In this process, yeast cells are destroyed, thereby eliminating fermentation and delaying the crystallisation process (Soares, Amaral, Oliveira, & Mafra, 2017). Cooling protects honey's natural colour, flavour, enzyme content and other biological substances, such as antioxidants (Eshete & Eshetie, 2019b). As enzyme activity of diastase, invertase and HMF are freshness indicators, it can be used to determine heat induced defects and type of length of storage (Soares, Amaral, Oliveira, & Mafra, 2017). During thermal processing, uncontrolled heating can cause the HMF content to increase and enzymatic activity to decrease. After pasteurisation the diastase activity and HMF are not affected, but invertase is impaired (Eshete & Eshetie, 2019b).

2.6 Honey adulteration

Single agricultural food products, including honey, are specifically vulnerable to food adulteration. Adulteration is a fraudulent practice which is defined as deliberate and intentional substitution, addition, tampering or misrepresentation of food for economical gain (Alghamdi, et al., 2020). Honey with its more than 200 major and minor components, as well as its high food and economic value, makes it an easy target for adulteration (Johari, Ashaari, Mamat, & Muhamad, 2019). Due to the high price value of monofloral honeys, they are prone to be adulterated. Monofloral honey has been identified as the sixth most adulterated food on earth (Alghamdi, et al., 2020). This has a global negative impact on the economy, health benefits and nutritional composition of food products.

The adulteration of honey is a good example of food fraud practise in the food industry. Various honey laundering schemes were uncovered during 2011 (Isopescu, Josceanu, Colta, & Spulber, 2017). For example, Chinese honey was transhipped through Germany, labelled as German honey and then exported to the USA by various suppliers. In New Zealand, Manuka honey is often mislabelled (He, Gkantiragas, & Glowacki, 2018). In 2018, honey being sold on the South African market, was identified as fake honey. Adulteration of honey can be classified as direct and indirect and adulteration due to blending. Direct adulteration is the simplest form and entails the addition of sugar syrups to the honey to increase the sweetness, while indirect adulteration occurs when honeybees are overfed during the main nectar period with honey, and chemical and industrial sugar solutions, to increase honey production. Blending occurs when pure honey is mixed with cheap and low-quality honey (Fakhlaei, et al., 2020).

Direct adulteration occurs when sugars e.g., sucrose, sugar beet, corn syrup (CS), high-fructose corn syrup (HFCS), high-fructose inulin syrup (HFIS), industrial sugars, invert sugar syrup (ISS) (cane sugar syrup and beet syrup) and maltose syrup are added to honey. The choice of adulterant is mostly dependent on the cost, availability and preference of the beekeeper (Se, Wahab, Yaacob, & Ghoshal, 2019; Fakhlaei, et al., 2020). In China, rice and maltose syrup are used as adulterants due to the difficulty in detecting sugars produced from C_3 plants (Eshete, 2019a; Se, Wahab, Yaacob, & Ghoshal, 2019; Fakhlaei, et al., 2020). Addition of sugars influences the physico-chemical parameters of honey e.g., electrical conductivity, enzymatic activity, and the glucose, sucrose, fructose and HMF contents (Soares, Amaral, Oliveira, & Mafra, 2017). Direct adulteration can also be done by adding water, processing and mislabelling of botanical and geographical origin (Jaafar, et al., 2020; Tsagkaris, et al., 2021).

Indirect adulteration includes harvesting prior to maturity/ripeness, overfeeding the honeybees, and misuse or overuse of veterinary drugs in the incorporation of sugars into honey via bee-feeding. Indirect adulteration is difficult to detect (Fakhlaei, et al., 2020; Jaafar, et al., 2020). C_3 (plants with a Calvin-Benson photosynthesis cycle) and C_4 (plants with a Hatch-Slack photosynthesis cycle) plants are used for indirect adulteration. Examples of C_3 plant sources are rice, wheat and beet, while C_4 plant sources are maize and sugarcane. C_3 plant nectar is preferred by honeybees over the nectar of C_4 plants (Eshete, 2019a). Moreover, the carbon isotope ratios vary in C_3 and C_4 plants depending on the type of photosynthetic cycle. The different carbon isotope ratios enable the identification and quantification of adulteration in commercial honey (Eshete, 2019a).

Adulteration has, however, became more advanced during the last decades. The manufacture of sugars that imitate the sugar composition characterising honey has become a general practise due to the difficulty in detecting sugars produced from C_3. Furthermore, low levels of C_4 sugar adulteration and especially addition of C_3 sugars are difficult to detect. Chinese beekeepers use rice and maltose syrup as adulterants, while Turkish and French beekeepers use wheat and rice as adulterants and in the other European countries, HFIS is used (Se, Wahab, Yaacob, & Ghoshal, 2019). During the blending of honey, high quality honey is mixed with inexpensive honey or synthetic honey that is of lower quality (Se, Wahab, Yaacob, & Ghoshal, 2019; Fakhlaei, et al., 2020). In China and Venezuela acacia honey is mixed with inexpensive rape honey. Acacia honey has a mild taste, a light yellow colour and does not crystallise, whilst rape honey is sweeter, light amber in colour and crystallises more rapidly (Fakhlaei, et al., 2020). Honeydew honey is adulterated by the addition of commercial low value nectar honey (Seraglio, et al., 2019).

There are several possibilities to determine and report adulteration. Adulteration can be detected if certain characteristics of the honey exceed the legally defined standards mentioned in the various regulatory frameworks e.g., the APS Act, in South Africa (DALRRD, 2000). Continuous food monitoring using scientific methods is, therefore, necessary to preserve the quality of food products. Techniques for assessing honey purity include sugar profiling, sensory analysis and physico-chemical analysis (Johari, Ashaari, Mamat, & Muhamad, 2019). At the moment, however, there is a lack of harmonisation of standards and methods globally, making it difficult to determine and report adulterations (Fakhlaei, et al., 2020).

2.7 Analytical techniques and methods used for tracing authenticity of honey

There are quite a number if analytical methods and approaches (Figure 2.7) used to authenticate honey with regard to botanical, geographical and entomological origin, as well as adulteration (de Almeida-Muradian, et al., 2020). Identification of pure honey and its authenticity was originally done with classical methods (pH-value, sugars, proline, enzyme activity (e.g., diastase and invertase), moisture and ash contents, acidity (free acid and lactone), HMF and sensory analysis (aroma, flavour and colour), which were based on honey properties (Chin & Sowndhararajan, 2020). Subsequently, specific methods and techniques where developed based on physical, chemical and biological properties of honey and are used to characterise and validate the quality of honey in terms of standard regulations (Johari, Ashaari, Mamat, & Muhamad, 2019). Due to the diversity of honey, it is important to continuously improve and modify these methods. Flowers, seasons, geographical and climatic conditions, bee species and sophisticated adulteration processes contribute to the diversity of methods used (Chin & Sowndhararajan, 2020).

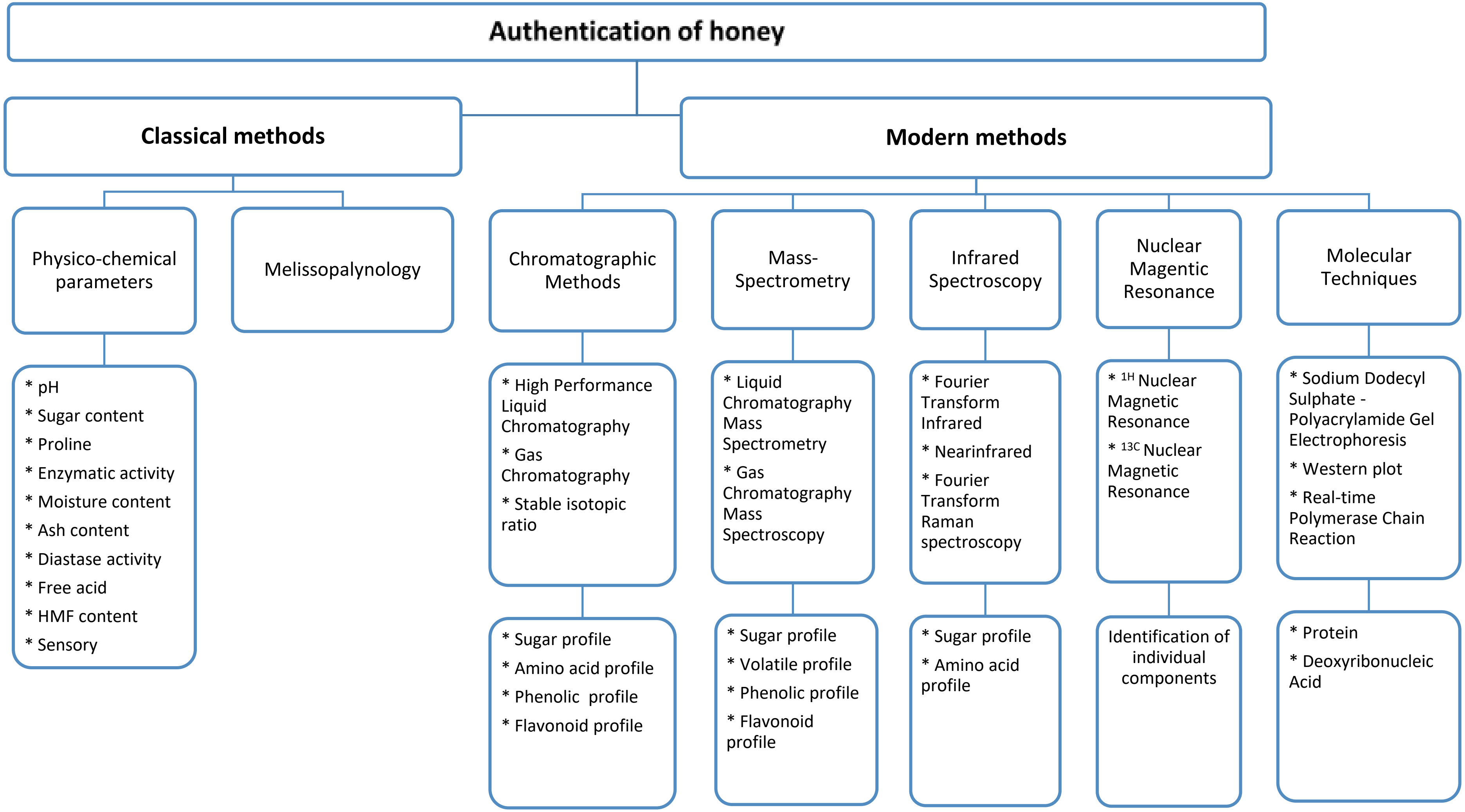

Figure 2.7: Classical and modern analytical methods determining authenticity of honey, (Adapted from (Chin & Sowndhararajan, 2020)

In Figure 2.8, the main factors contributing to honey authentication are depicted (Soares, Amaral, Oliveira, & Mafra, 2017). In the past, the focus was on physico-chemical parameters which were used to determine the botanical origin of honey. This includes microscopic analysis of pollen, determination of pH-value, sugars, proline, enzyme activity (e.g., diastase and invertase), moisture and ash contents, acidity (free acid and lactone), HMF and sensory analysis (aroma, flavour and colour) (Chin & Sowndhararajan, 2020).

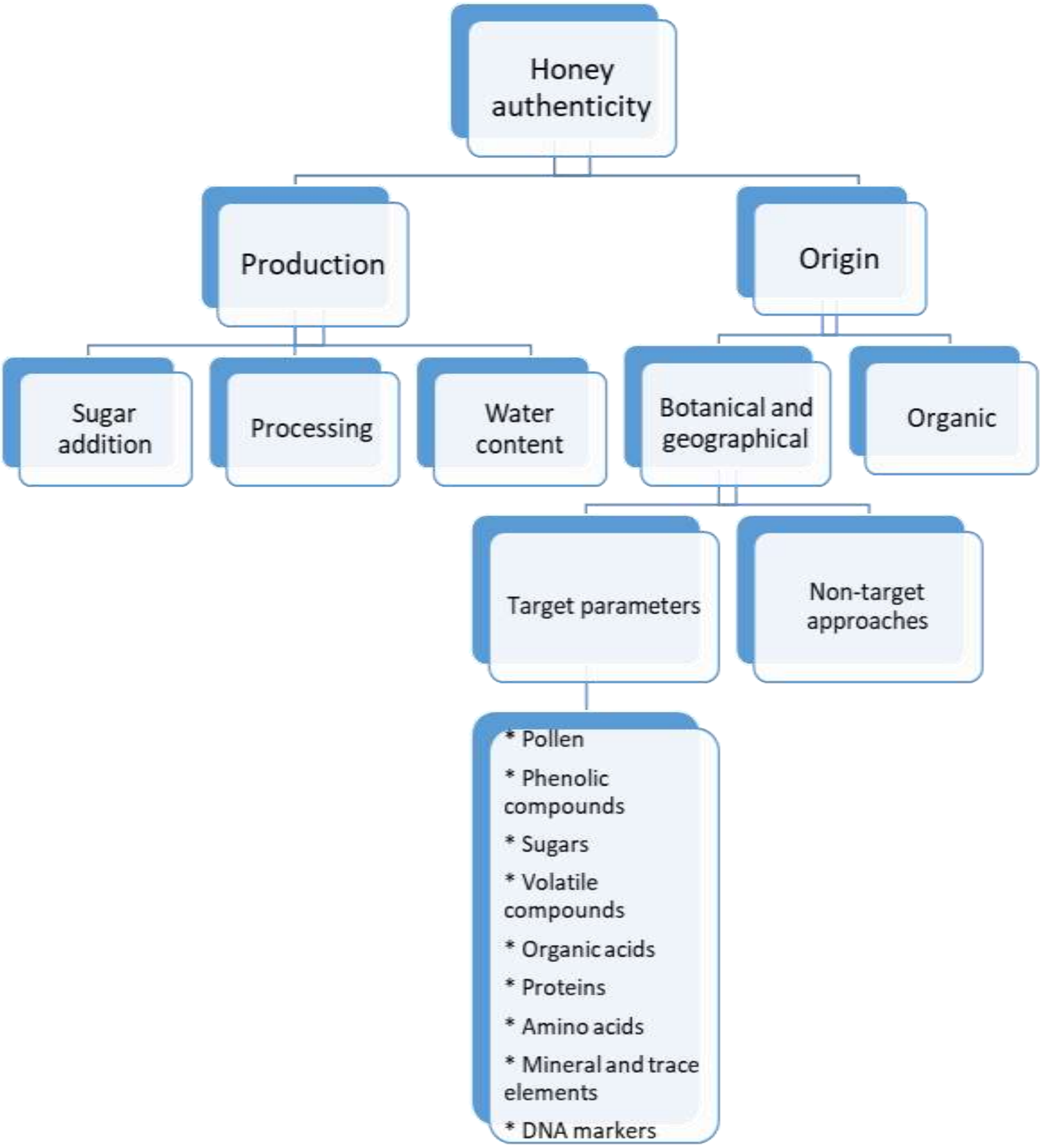

Figure 2.8: Schematic representation of the factors determining authenticity honey, (Adapted from (Soares, Amaral, Oliveira, & Mafra, 2017)

Microscopic analysis of pollen (melissopalynology) is used to determine the floral and geographical origins of honey. This is also a useful screening method for detecting cane sugar adulteration, but it is currently not effective for detecting the synthetically prepared syrups. It is based on the detection of specific markers such as epidermal cells and sclerous rings that indicate the addition of cane sugar (Tura & Seboka, 2019). Another limitation of melissopalynology is that it cannot detect the addition of pollen (adulteration) or inadequate filtration of honey by beekeepers (Soares, Amaral, Oliveira, & Mafra, 2017).

Sensory analysis (favour, colour, taste) is used to confirm quality, verify the absence of defects e.g., fermentation, odours and impurities, as well as to determine the botanical origin of honey. In a study done by Schievano and co-workers (2015) on Ecuadorian commercial honey, honey made by *A. mellifera* had a characteristic floral taste whereas the fake honey had a candy-like odour/aroma (Schievano, Zuccato, Finotello, & Vit, 2015). However, both the microscopic and sensory analysis require skilled analysts and extensive training to perform. Sensory analysis is also subjective and thus there is variability in human perception with regard to quality parameters.

Microscopic analysis is also slow and tedious (Danezis, Tsagkaris, Camin, Brusic, & Georgiou, 2016). Due to the limitations of the classical methods, more modern analytical methods are used to authenticate honey in terms of their botanical and geographical origin. These include: measuring sugar, aroma and amino profiles, mineral content, and phenolic and flavonoid compositions using techniques such as chromatographic-, mass spectrometry-based-, vibrational spectroscopy (infrared-based, near Infra-Red and Raman) techniques, nuclear magnetic resonance (NMR) stable isotope analysis, flame ionisation detectors (FID) and sensor array (Chin & Sowndhararajan, 2020).

Chromatographic techniques are known to be powerful tools and are currently the most frequently used methods for authenticating food products (Tsagkaris, et al., 2021). A variety of chromatographic methods have been developed to authenticate honey. Authentication by chromatography is based on the profile of specific compounds. It can be used to determine the adulterants, and the botanical and geographical origin of honey. Chromatographic techniques include liquid chromatography (LC), gas chromatography (GC) and thin layer chromatography (TLC). Liquid chromatography is used to determine protein, amino acids, sugars, vitamins, phenolic compounds, triglycerides, chiral compounds and pigments, while GC is used for the analysis of volatile and semi-volatile (aroma) compounds (Trifković, Andrić, Ristivojević, Guzelmeric, & Yesilada, 2017; Abbas, et al., 2018).

Liquid chromatography and GC can also be used for tracing adulteration in honey. Liquid chromatography can identify C_3 (e.g., rice syrup) and C_4 (adulterants). In addition, GC can detect adulterants HFCS, CS, HFIS and inverted sugar (IS). The detection of HFCS and ISS is mainly based on

Difructose anhydrides (DFAs) that are formed during caramelisation (Se, Wahab, Yaacob, & Ghoshal, 2019). Thin layer chromatography has not been widely used in the past for honey authentication and no TLC studies have been done on the determination of botanical and geographical origin of honey. Thin layer chromatography has only been used to detect adulterants e.g., HFCS, rice syrup and beet sugar (Se, Wahab, Yaacob, & Ghoshal, 2019). In addition, contaminants, HMF levels, pesticides, antibiotics and their metabolites can also be detected by this technique. Compared to GC and High-performance liquid chromatography (HPLC), TLC has a high sample throughput, is a rapid low-cost analysis with increased precision and accuracy if automated (Trifković, Andrić, Ristivojević, Guzelmeric, & Yesilada, 2017).

The high demand for authentication of food products, such as honey, contributes to the many studies that have been done to determine the botanical and geographical origin of honey by using chromatographic techniques combined with mass spectrometry (MS) (Chin & Sowndhararajan, 2020). Liquid chromatography mass spectrometry (LC-MS) and gas chromatography mass spectroscopy (GC-MS) can also be used in determining the botanical and geographical origin of honey. Liquid chromatography mass spectrometry is also a rapid and reliable means used to detect adulteration and used to characterise phenolic compounds (Trifković, Andrić, Ristivojević, Guzelmeric, & Yesilada, 2017).

Stable carbon isotope ratio analysis can be used to differentiate honey from different floral and geographical origins and identify adulteration (Chin & Sowndhararajan, 2020). The principle of this technique is based upon the difference in isotope ratio ($^{13}C/^{12}C$) of C_3, C_4, and Crassulacean Acid Metabolism (CAM) plants photosynthesis cycle (Eshete, 2019a). In the determination of the geographical origin of honey, climatic conditions and agricultural practices are constraints because they influence the ^{13}C values of honey (Se, Wahab, Yaacob, & Ghoshal, 2019). In combination with other techniques, stable carbon isotope technique it has become a powerful tool in the determination of the origin of honey and also as an indicator of organically grown food (Hohmann, et al., 2015).

Isotopic differences can also be diagnostic of adulteration e.g., in honey adulteration low-cost sugar syrups from C_4-plants (e.g., cane sugar and corn syrup) can be detected (Chin & Sowndhararajan, 2020). Most of the plants that honeybees use as nectar sources are C_3 plants. C_4 plants, such as maize/corn and sugarcane are not usually part of a honeybee’s diet and can, therefore, be detected. However, if honey is adulterated with sugars that have the same isotopic ratio as honey e.g., beet, rice or wheat, this method is unable to detect these adulterants (C_3 plants) (Trifković, Andrić, Ristivojević, Guzelmeric, & Yesilada, 2017). All plants have their own $^{13}C/^{12}C$ isotope ratio values, C_3 plants range from -21‰ to -32‰, C_4 plants range from -12‰ to -19‰, respectively (Se, Wahab, Yaacob, & Ghoshal, 2019). Pure honey has a $\delta^{13}C$ value of -23 ‰ or lower. Honey with $\delta^{13}C$ values in the range of -23.5‰ to -21.5‰ falls into the grey area, i.e., the honey could be adulterated or not. Honey samples with a $\delta^{13}C$ value of -21.5‰ or higher are regarded as adulterated (Eshete, 2019a). However, false positives can occur if honey is made predominantly from CAM plants ($\delta^{13}C$ values -11‰ to -13.5‰). These plants can have the same type of photosynthetic pathway as a C_3 or C_4 due to environmental conditions, such as availability of water (Vetrova, Kalashnikova, Melkov, & Simonova, 2017). Indirect adulteration by overfeeding bees with corn or sugar cane during nectar flow is also an adulteration practice than can be detected (Soares, Amaral, Oliveira, & Mafra, 2017). If honey is naturally made from C_4 plants, false positives can occur for sugar adulteration or because of low protein fractions e.g., in acacia and lavender. To detect adulteration with added HFCS in honey, Stable Carbon Isotopic Ratio Analysis (SCIRA) and protein fraction evaluation should be performed (Se, Wahab, Yaacob, & Ghoshal, 2019).

Recent studies indicated that nuclear magnetic resonance (NMR) in conjunction with chemometrics is a valuable tool that can be used in food authentication (Chin & Sowndhararajan, 2020). As this technique is independent of pollen manipulations, it can be used for classifying the botanical (floral) and geographical origins of honey (Spiteri, et al., 2017; Trifković, Andrić, Ristivojević, Guzelmeric, & Yesilada, 2017). This technique produces a unique fingerprint profile for each honey which can assist in traceability. Nuclear magnetic resonance has been used to characterise Manuka honey markers, methylglyoxal, dihydroxyacetone and leptosperin (Spiteri, et al., 2017). By using quercitol as a biomarker, NMR could also differentiate oak honeydew honey from other type of honeydew honey (Jakubik, Borawska, & Socha, 2020). In true Ecuadorian honey, NMR detected the fluorophores proline, tyrosine, and phenylalanine, which were absent in fake honey (Schievano, Zuccato, Finotello, & Vit, 2015). Adulteration with commercial sugar syrups, for example HFCS, can also be determined with the aid of NMR (Chin & Sowndhararajan, 2020). Currently studies are being done to differentiate between conventional and organic honey (Consonni, Bernareggi, & Cagliani, 2019). The advantages of NMR over the other spectroscopic techniques, are its high reproducibility, sensitivity and rapid data acquisition. However, NMR is also an expensive technique (Se, Wahab, Yaacob, & Ghoshal, 2019). The majority of techniques reported for honey analysis (e.g., physico-chemical characterization, biological and therapeutic evaluation) or detection of adulterants are slow, laborious, tedious, and destructive. In addition, they require expensive equipment and skilled operators and are not suitable for rapid screening (Ferreiro-González, et al., 2018).

Spectroscopic technology has been used in the food industry, together with chemometrics, to monitor and evaluate the composition of foods, and it represents a good alternative to the more commonly used methods to authenticate food products (Ferreiro-González, et al., 2018; Tura & Seboka, 2019;

Chin & Sowndhararajan, 2020). These methods provide the simultaneous determination of sugars and other physico-chemical parameters and can be used for routine quality control of honey. Near-Infrared spectroscopy (NIR), visible spectroscopy, infrared spectroscopy (IR) and Raman spectroscopy in combination with chemometrics (e.g., principal component analysis (PCA), hierarchical cluster analysis (HCA), linear discriminant analysis (LDA) or partial least squares regression (PLS)) have been successfully applied in the authentication of honey to determine the botanical, geographical origin and detection of adulteration (Soares, Amaral, Oliveira, & Mafra, 2017; Abbas, et al., 2018). Various adulterants were identified by using the chemometric models together with the spectroscopic technique (Se, Wahab, Yaacob, & Ghoshal, 2019). Examples of adulterants which were detected, were HFCS, maltose syrup (MS), fructose, glucose, (inverted sugar), hydrolysed inulin syrup and malt (Ferreiro-González, et al., 2018).

Due to its many advantages, namely rapidity, lower cost, and its non-destructive, and environmentally friendly and user friendly properties, spectroscopy can be used as a screening method in routine quality control of commercial honey (Trifković, Andrić, Ristivojević, Guzelmeric, & Yesilada, 2017; Ferreiro-González, et al., 2018; Tura & Seboka, 2019). The various limitations of spectroscopy are that samples can be destroyed due to prolonged exposure to the laser beam, and that the accuracy of the prediction model depends on the number of samples used to generate the dataset and the variation in spectra, as well as which components are being measured (Trifković, Andrić, Ristivojević, Guzelmeric, & Yesilada, 2017; Chin & Sowndhararajan, 2020). In addition, the standalone spectroscopic techniques, such as Raman, NIR and Mid Infrared (MIR) spectroscopy cannot identify adulterant without using chemometrics data analysis (Se, Wahab, Yaacob, & Ghoshal, 2019). Chemometric analysis is widely applied to the detection method as a helpful tool for the reduction of sample complexity and classifies samples into groups based on their similarities (Fakhlaei, et al., 2020).

Biomolecular methods are being used more frequently as deoxyribonucleic acid (DNA)-based markers are recognised as highly efficient and accurate to detect botanical, geographical, entomological origin and adulteration (Jakubik, Borawska, & Socha, 2020). Hence, these molecular techniques (genomic and proteomic) are complementing and starting to replace earlier methods (Abbas, et al., 2018). Protein based methods represent techniques that use proteins as specific markers and include, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), Western-blot and real time Polymerase Chain Reaction (PCR). Honey contains approximately 0.2% protein which originates from the nectar of plants and bees and these proteins appear in the form of enzymes which have different molecular weights depending on the bee species. Protein characterisation can be used as a chemical marker for botanical classification, but also to differentiate between honey produced by different honeybee species *(A. mellifera* and *A. cerana)* (Chin & Sowndhararajan, 2020).

Furthermore, DNA-based methods can be used in the identification of the origin of honey, since honey (a sugary solution) contains DNA of bee and plant (pollen) origin (Jakubik, Borawska, & Socha, 2020). Species-specific DNA markers from pollen of *Leptospermum* have been used to authenticate Manuka honey (Moar, 1985; McDonald, Keeling, Brewer, & Hathaway, 2018). Sobrino-Gregorio et al., (2019) used conventional and real time PCR DNA amplification to detect and quantify adulteration of honey with rice syrup (C_3 sugar) (Sobrina-Gregorio, Vilanova, Prohens, & Escriche, 2019). Deoxyribonucleic acid-based markers are recognised and accepted as highly efficient and accurate identification methods. However, the majority of these molecular tools provide qualitative assessment, rather than a quantitative estimate of adulteration (Abbas, et al., 2018). In addition, the performance, implementation and official control of DNA-based tests is limited. The limitations include the low amount of pollen present in honey, the fact that the complexity of the honey matrix may influence the DNA extraction, furthermore, the DNA based tests will not be able to identify if the honey (sugary solution) is adulterated with desired pollen by the producer (Soares, Amaral, Oliveira, & Mafra, 2017).

Other techniques include Differential Scanning Calorimetry (DSC), which can discriminate between pure and adulterated honey (Chin & Sowndhararajan, 2020). Biosensor technology, such as electronic nose (e-nose) and electronic tongue (e-tongue) analyses, together with chemometrics, which are used to differentiate honey of different botanical and geographical origins (Se, Wahab, Yaacob, & Ghoshal, 2019). The e-nose and e-tongue methods employ non-specific arrays that mimic human senses. Sensory analysis (e.g., aroma and taste) can provide complementary information to the physio-chemical parameters and enhance the speed and cost efficiency of food analysis (Tsagkaris, et al., 2021).

The methods of choice in food laboratories are still the targeted approach methods that determine more specific compounds that are time consuming and expensive, but have a high accuracy, sensitivity and selectivity. While non-targeted approaches are less expensive and less sensitive, they are rapid, non-destructive and can be used as screening tools (Abbas, et al., 2018). Conventional target analysis is where a specific marker compound is searched for, identified and measured to determine if its concentration exceeds the set limit value (Esslinger, Riedl, & Fauhl-Hassek, 2014). The search for specific markers limits this technique, because the researcher must first identify the marker to be used and then determine if there is/are method(s) available to detect that specific marker before the method can be used for detection purposes. Techniques that are used in target analysis to authenticate honey are the C-isotope approach, and gas and liquid chromatography (Ferreiro-González, et al., 2018). Unfortunately, the producers and manufactures of honey know about these types of analysis and how to avoid detection of adulterants (McGrath, et al., 2018). It is evident that the increase in fraudulent practices of honey adulteration intensified the need for more rapid, simple, cheap and reliable techniques for screening purposes. In the more recent methods, an approach of non-targeted analysis is followed, e.g., spectroscopic methods and nuclear magnetic resonance (NMR) in conjunction with statistical analysis. In this analysis the fingerprint profile of the sample is determined and measured against different profiles recorded in a database for that type of food sample (Ferreiro-González, et al., 2018). The application of these screening methods is still limited in routine quality control to determine botanical origin, geographical origin, adulteration and chemical characteristics of honey (Soares, Amaral, Oliveira, & Mafra, 2017).

In summary, analytical testing is a valuable tool to authenticate honey. However, in the case of honey, the diversity of honey contributes to the limitations of the usage of analytical testing to examine, detect and verify all types of food fraud. Once food fraud is detected and tested for routinely, the fraudsters will move on to another fraudulent practice. Consequently, a combination of techniques is required to address the challenges of false declarations regarding geographic and botanical origin, adulteration and the quality of honey.

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CHAPTER 3: MONITORING THE QUALITY OF HONEY: SOUTH AFRICAN CASE STUDY

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Highlights:

- *South African honey were evaluated using the Agricultural Standards Act as benchmark*
- *Evaluation of the quality parameters from local and imported honey over 10 years*
- *Comparing the physico-chemical parameters of honey from different floral origins*
- *On average 90% local and 92% imported honey is compliant*
- *Significant differences were found in honey from different floral origin*

3.1 Abstract

The popularity of honey as a high-valued commodity is growing and consequently, honey adulteration is on the rise affecting the honey quality. The quality of the honey on the South African market was evaluated using the Agricultural Product Standards Act, 1990 as assessment tool. Various physico-chemical characteristics were tested which indicated compliance of >80% for all honey samples. A canonical variate analysis using 95% confidence regions indicates significant differences between the quality of local and imported honey with total acid, sucrose and ash as the parameters mostly distinguishing between the groups. Honey produced from agricultural crops differed significantly from all other forage types. The parameters that mostly distinguished between forage types were Lund, hydroxy-methyl-furfural and ash content. Even though honey sold on the South African market is generally in accordance with national and international standards, an overall declining trend in quality was observed and it should be continuously monitored.

Keywords: *Honey, Quality parameters, Adulteration, Floral origin*

3.2 Introduction

Honey, as defined in the Codex Alimentarius Standards, is a natural product produced by honeybees from the nectar of flowers that contains no added foreign substances (Codex Alimentarius, 2001). It consists of various sugars (i.e., fructose, glucose, sucrose and maltose), water, trace elements, vitamins, proteins and organic acids. Honey is well known for its medicinal properties and sensory characteristics such as its unique aroma and sweet taste. The latter is due to the predominance of fructose (European Commission, 2002; Dezmirean, Marghitas, & Dezmirean, 2011; Zhou, Taylor, Salouros, & Prasad, 2018). Consumers generally perceive it as a healthier and natural sweetening alternative to table sugar (Kumar, Ansari, & Walia, 2018).

Honey is an authentic product with a unique chemical fingerprint determined by its production environment, e.g., geographical and botanical origin as well as processing procedures (European Commission, 2002). The rich diversity of plant species in South Africa includes agricultural crops, forestry, natural forage, vegetation units, weeds and suburban plants, which are pollen and nectar sources for bees during honey production (Guelpa, Marini, du Plessis, Slabbert, & Manley, 2017). Currently, South Africa can only produce 50% of the market demand for honey (2000 tons). To compensate for this shortage, South Africa imports honey from various countries including China, Argentina and Romania (IDC, 2016). South Africa exports limited amounts of honey mostly to African countries including Namibia (41%), Botswana (26%), Lesotho (10%) and Zimbabwe (9%). South Africa is ranked 57th in the world for honey exports and imports (IDC, 2016). In South Africa, approximately 50% of honey produced originates from *Eucalyptus* spp., followed by citrus, fynbos and sunflower (Masehela, 2017). The physico-chemical composition of honey is determined by various parameters including its sugar content (i.e., fructose, glucose, sucrose and maltose), pH, total acidity, free acid, lactone, moisture, ash, specific rotation, protein and hydroxyl-methyl-furfural (HMF) content (Bogdanov, et al., 1999; DAFF, 2000; Codex Alimentarius, 2001). The compilation of specific standards describing the physico-chemical composition of honey is pivotal for the characterization and evaluation of its chemical composition and regulation of possible adulteration (Codex Alimentarius, 2001).

Honey is an easy target for fraud, which entails the addition of adulterants such as sugar syrups, molasses, and natural syrups such as maple syrup to pure honey to increase yield for economic gain (Guelpa, Marini, du Plessis, Slabbert, & Manley, 2017). Adulterated honey has been on the increase, particular with sugars such as fructose (40%), glucose (30%), maltose (8%) and sucrose (2%). Another fraudulent practice is the masking of botanical and geographical origin (Olawode, Tandlich, & Cambray, 2018). In a study done by Zhou, et al (2018) examining 100 honey samples from 19 different countries showed that 27% of commercial honey samples tested were of questionable authenticity. Of this adulterated honey, 52% were from Asia, 28% from Europe and 18.4% Australia. Various adulterated honey complies with a set of quality criteria suitable for consumer usage. It, however, fails as an authentic product as it contains added substances (Paiva, 2013). The continuous monitoring of the quality of food products on the market is required to ensure the production of authentic products.

Regularly revised qualitative and quantitative tools are necessary in the global and national market to implement an effective food control system. The assessment tool used to evaluate the quality of honey available on the South Africa market is the Agricultural Product Standards (APS) Act 119 of 1990, 2000 (DAFF, 2000). According to the APS Act, all animal and processed products produced locally or imported should be analysed for compositional requirements. The combination of analytical techniques, standards and legislation determines the efficiency of law enforcement to ensure the quality of different products. These legislations are according to international standards such as the International Honey Commission (IHC) and Codex Alimentarius Honey Standards (Bogdanov, et al., 1999; Codex Alimentarius, 2001).

The purpose of this study was to evaluate and compare the quality of imported and locally produced honey available on the South African market by analysing the data generated by the Department of Agriculture, Forestry and Fisheries (DAFF), in South Africa from 1998 to 2017. Honey is perceived as an easy target for adulteration and an escalation in the adulteration practices is reported worldwide, therefore, it is hypothesised that more than 20% of the honey on the South African market may not comply with the APS Act. Chemical composition can play a role in the authentication of honey, therefore the chemical composition of honey samples from different floral sources e.g., agricultural crops, forestry, indigenous genera vegetation units and mixed flora on the local market were also compared.

3.3 Materials and Methods

3.3.1 Sampling

To evaluate the quality of imported and locally produced honey from different regions in the country, honey, 857 (local: n=638; imported: n=70 and unknown n=149) honey samples available to consumers on the South African retail market were analysed over a period of 19 years from 1998 to 2017 as part of the monitoring program of the quality of honey by the Department of Agriculture, Forestry and Fisheries (DAFF) in South Africa. Samples were randomly selected by DAFF food inspectors at retail level and sent to the sub-directorate National Analytical Services of DAFF for analyses. Local samples (n=638) originated from all provinces in South Africa namely: KwaZulu-Natal (NTL) (n=103), Northern Cape (NC) (n=6), Eastern Cape (EC) (n=38), Gauteng (GP) (n=258), Free State (FS) (n=19), North West (NW) (n=19), Limpopo (LIM) (n=30), Mpumalanga (MP) (n=44), unknown origin (UNK) (n=149), Western Cape (WC) (n=121) and imported (IMP) (n=70). Imported samples originated from China (n=13), Argentina (n=7), Romania (n=5), India (n=4), Zambia (n=4), Egypt (n=3), Kuwait (n=3), Lesotho (n=2), Singapore (n=1), Australia (n=1), New Zealand (n=1), Zimbabwe (n=1) and from unknown origin (n=25). Towards identifying the different quality groupings of honey locally produced, a subset of 382 honey samples of known floral sources were selected from the original samples. This subset of samples originated from agricultural crops (n=139): avocado, canola, citrus, grape, kidney blossom, litchi, lucerne, macadamia, mango, onion and sunflower; forestry (n=106): eucalyptus; indigenous genera (n=22): acacia, aloe and wag-‘n-bietjie; vegetation units (n=76): field, forest, fynbos, indigenous, mixed flora, wildflower, polyflora; and mixed flora (n=39): polyflora were selected for analyses.

3.3.2 Evaluation of the physico-chemical properties

All samples were analysed in duplicate using methods recommended by the Official Methods of Analysis (AOAC), the International Honey Commission (IHC) and the Codex Alimentarius Honey Standards (AOAC, 1995; Bogdanov, et al., 1999; Codex Alimentarius, 2001). All samples were analysed by the National Analytical Services, Directorate Food Safety and Quality Assurance, Department of Agriculture, Land Reform and Rural Development in Pretoria. The physico-chemical parameters tested were sugars (fructose, glucose, sucrose and maltose), reducing sugars (sum of fructose, glucose and maltose), fructose/glucose ratio, pH, total acidity (free acid and lactone), moisture, ash, Lund’s precipitate and hydroxy-methyl-furfural (HMF) (DAFF, 2000). Although specific rotation is not defined by either IHC or Codex, it was determined in this study as an additional quality parameter.

Methods is validated by the laboratory every five years. Accuracy was verified through the Food Analysis Performance Assessment Scheme (FAPAS) that included both inter- and intralaboratory proficiency schemes (FAPAS, 2019). However, the physico-chemical parameters tested depends on the availability of such tests, hence, only sugars (fructose, glucose and sucrose), pH, free acid, moisture and HMF accuracy were verified through FAPAS. Intra-laboratory tests where the same samples were analysed by different analysts in the same laboratory were used as verification for the rest of the analyses. Available validation data, including measurement uncertainty is summarised in Table 3.1. Performance in a FAPAS proficiency test is considered fit for purpose when a z-score lies within a range of a ±2 (Fera Science Ltd, 2016). Relative Standard deviation (RSD) <5% were acceptable for intra-laboratory analyses (Fera Science Ltd, 2016). Relative standard deviation (RSD) <5 % were acceptable for intra-laboratory analyses.

Table 3.1: Summary of proficiency and intra-laboratory testing

Physico-chemical parameter	Standards and requirements for compliance		
	FAPAS	Intra-laboratory testing	Measurement Uncertainty
	Z-score (-2≤ z ≤2)	<5% Relative standard deviation (RSD)	
Sucrose (%)	-0.3 ≤ z ≤2.0		
Fructose (%)	-1 ≤ z ≤1.7		
Glucose (%)	z ≤2.0		
pH-Value	z ≤2.0		
Hydroxy-methyl-furfural (mg HMF/kg)	-2.0≤ z ≤2.0		X ± 0.81
Moisture (%)	-0.4≤ z ≤2.0		X ± 1.10
Free acid (meq/kg)	-0.5 ≤ z		
Reducing sugars (%) (Sum of fructose, glucose and maltose)		<4%	
Ratio of fructose:glucose		<5%	
Total acid (meq/kg)		<3%	
Lund (cm^3)		<5%	X ± 1.09
Specific rotation (°)		<3%	
Ash (%)		<2%	X ± 0.01

FAPAS=Food Analysis Performance Assessment Scheme

3.3.2.1 Sugars

All chemicals are of analytical grade (>95%) unless otherwise specified. A High-performance Liquid Chromatograph (HPLC) Hewlett Packard 1100 equipped with a Refractive Index detector (Chemetrix) thermostated at 30°C was used to analyse the fructose, glucose, sucrose and maltose content. Degassed acetonitrile:water (80:20) mobile phase and a flow rate of 1.50 ml/min was used for separation. A diluted honey solution containing 5 g of honey dissolved in 100 ml distilled water was prepared and filtered with a 0.45 µm polytetrafluoroethylene (PTFE) filter. Thereafter, 5 µl of diluted honey solution was injected onto a Supelcosil LC-NH_2 HPLC column (250 mm length x 4.6 mm diameter, 5 µm particle size). A 1% (99% purity) sugar standard mix containing fructose, glucose, sucrose and maltose was used as standard bought from Sigma-Aldrich. ChemStation Software wasused for identification and quantification of sugars, which were reported as a percentage (%) (Bogdanov, Martin, & Lullman, 1997).

3.3.2.2 pH and total acidity

The pH was measured with a Eutech CyberScan PCD 6500 pH-meter (Heyns Lab Supplies). An acid-base titration was used to determine the total acidity of each honey sample. For this analysis, 10 g of honey sample was dissolved in 75 ml carbon dioxide free, distilled water in a 250 ml beaker using a magnetic stirrer. The pH-meter was calibrated prior to analyses with standard pH-buffers (Heyns Lab Supplies) with a pH of 4, 7 and 10 respectively. The free acid of each honey sample was then determined by a direct titration of each honey solution with 0.05 N Sodium hydroxide (NaOH) to pH 8.3. This was followed by the addition of 10 ml (excess) 0.05 N NaOH which was immediately titrated back to pH 8.3 with 0.05 N Hydrochloric acid (HCl) to determine the lactone content (lactonic acidity). A blank test was done by titrating 75 ml of carbon dioxide free water with 0.05 N NaOH to a pH 8.3 (AOAC, 1995a).

The free acid and lactone content were subsequently used to calculate the total acidity. Calculations for total acidity were as follows:

$$\text{Free acid (meq/kg)} = (\text{ml NaOH titrated} - \text{ml blank}) \times 50/\text{sample mass (g)}$$

$$\text{Lactone (meq/kg)} = (10 - \text{ml HCl titrated}) \times 50/\text{sample mass (g)}$$

$$\text{Total acidity (meq/kg)} = \text{Free acid} + \text{Lactone}$$

3.3.2.3 Moisture content

The moisture content was determined using Refractive Index (RI) in combination with the Chataway table. An automatic digital refractometer, Atago RX-5000α (Bashumi Instruments & Control Services), calibrated with distilled water was used for the measurement. A drop of honey was placed on the surface of the prism and a refractive index reading was taken at 20°C and converted to a percentage (g/100 g) using the Chataway table (AOAC, 1995b).

3.3.2.4 Ash content

The ash content was determined by the gravimetric method described by Liberato, Morais, Magalhaes, Magalhaes, Cavalcanti, Silva (2013). Firstly, a platinum crucible was dried, cooled in a desiccator and weighed ±0.001 g (M_2). Thereafter, approximately 5±0.001 g (M_0) of honey was weighed into the platinum crucible and heated on a hotplate in a fume cupboard until carbonised. It was then incinerated overnight in a muffle furnace (Heyns Lab Supplies) at 600°C to a constant weight. The platinum crucible was subsequently cooled in a desiccator and immediately weighed ±0.001 g (M_1) (Liberato, et al., 2013). The percentage ash was calculated using the following equation:

$$\text{Ash\%} = (M_1 - M_2)/M_0 \times 100$$

Where: M_0 = nominal weight of honey

M_1 = weight of crucible + ash

M_2 = weight of crucible

3.3.2.5 Lund's reaction

Approximately 5 g honey sample was weighed and dissolved in 50 ml water. Thereafter, 20 ml of this solution was transferred to two separate 100 ml crow receivers. This was followed by the addition of 5 ml tannic acid (0.5%) (Heyns Lab Supplies) to one of the crow receivers. This solution was then diluted with distilled water to a volume of 40 ml. Subsequently, 5 ml of tungstophosphoric acid (2%) (Heyns Lab Supplies) was added to the second crow receiver, which was also diluted with distilled water and made up to a volume of 40 ml. Both mixtures were then shaken, closed and allowed to stand for 24 hours in a dark cupboard at room temperature. The tungstophosphoric and tannic acid mixtures were respectively used to precipitate nitrogenous compounds and albuminoids (protein content) within the honey samples. If a combined precipitate of more than 0.6 ml formed, the honey was considered to be pure (White, JW, 1957; de Almeida Muradian, et al., 2013; Salazar, de Freitas, da Luz, Bersch, & dos Santos Salazar, 2017).

Lund was calculated as:

Lund (protein precipitate) = Tannic acid precipitate + Tungstophosphoric acid precipitate

3.3.2.6 Hydroxy-methyl-furfural

White's spectrophotometric method as prescribed by the IHC was used to determine the HMF content (White, JW, 1957; International Honey Commission, (IHC), 2009). Approximately 5 g honey was diluted in 25 ml water to which 0.5 ml 15% Carrez 1 (Potassium Hexacyanoferrate (II)) (Heyns Lab Supplies) and 0.5 ml 30% Carrez 2 (Zinc Acetate) (Heyns Lab Supplies) reagents were added. Water was then added to a final volume of 50 ml in a volumetric flask and mixed. This solution was then filtered through Whatman No 1 filter paper. The first 10 ml of the filtrate was discarded. Aliquots of 5 ml honey solution were then transferred to two test tubes which contained either 5 ml distilled water (honey sample) or 5 ml 0.2% sodium bisulphite solution ($NaHSO_3$) (reference sample) respectively and mixed well before being transferred to 10 mm quartz cuvettes. The absorbency at 284 nm and 336 nm of both samples were respectively measured within an hour using an Agilent Cary 100 double-beam UV-Visible spectrophotometer (Chemetrix). Each quartz cuvette was filled with a honey solution or the reference solution respectively (International Honey Commission, (IHC), 2009). The spectrophotometer was calibrated with the reference solution. If the absorbance at 284 nm exceeded

0.6, the samples and blank reference was diluted with water and sodium bisulfite (Heyns Lab Supplies (60.3%)) 0.2% in the same proportion respectively. The following equation was used to calculate the HMF in mg/kg:

$$\text{HMF (mg/kg)} = (\text{Abs}_{284} - \text{Abs}_{336}) \times 149.7 \times 5 \times \text{Dilution factor/Weight of honey sample (g)}$$

Where:

Abs_{284} = absorbance at 284 nm Abs_{336} = absorbance at 336 nm

149.7 = Constant

3.3.2.7 Specific rotation

An automatic digital polarimeter, Atago AP-300 (Bashumi Instruments and Control Services), was used to determine the specific rotation of the respective honey samples. For this analysis, approximately 26 g of honey was dissolved in distilled water to which 5 ml of a clarifying agent (aluminium potassium disulfate [$KAl(SO_4)_2.12H2O$] / alumina cream) (Labchem) was added (AOAC, 1995c). This solution was then mixed by stirring it with a glass rod. It was then transferred into a 100 ml volumetric flask, made up to volume with distilled water and filtered through Whatman 2V filter paper into a 200 mm glass tube with a bubble trap. After the instrument was calibrated with distilled water, the tube containing the honey solution was placed into the instrument which took a rotation reading at 20°C (AOAC, 1995d).

3.3.2.8 Data analysis

All data regarding the quality of the local and imported honey was summarized in Excel and imported into GenStat (VSN International, 2017) for data evaluation. The data was filtered by removing all extreme outliers to prevent data skewing in the subsequent analyses (Krzanowski, 1988). The filtered dataset was used to perform a canonical variate analysis (CVA) to determine the quality groupings firstly of honey originating from various geographic regions and secondly of local honey produced from various forage types namely agricultural crops, forestry, indigenous genera, vegetation units and mixed flora. The variates used for the analyses were the nine standard honey quality parameters as determined by the APS Act. The logarithms of sucrose, total acid and HMF, as well as the square root of ash were used to stabilize variances and to normalise the data for both data sets.

3.4 Results and Discussion

The compliance of the honey samples to the APS Act standards (excluding specific rotation) ranged from 80% to 94% with a mean of 90% and 92% for the local and imported honey samples respectively. Amongst the local samples, the lowest compliance was found in the Northern Cape (NC) (80%)

whereas the highest was in Limpopo (LIM), North West (NW) and Free State (FS) (94%). Figure 3.1 shows the percentage (%) compliance for the local and imported honey samples. The high average compliance rate of the local (90%) and imported (92%) honey samples demonstrates that most of the honey sold on the South African market is in accordance with the APS Act (DAFF, 2000; Codex Alimentarius, 2001).

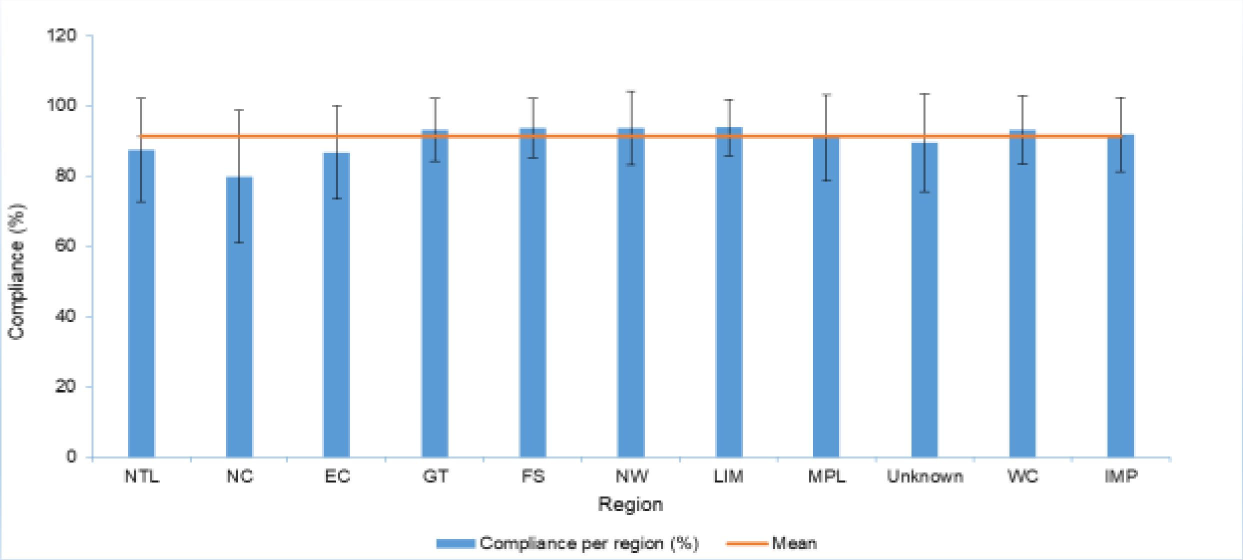

Figure 3.1: Compliance of locally produced and imported honey in South Africa for honey samples obtained from 1998 to 2017

Compliance of locally produced and imported honey in South Africa according to physico-chemical parameter requirements of the Agricultural Products Standards Act (Act 119 of 1990) is reported in Table 3.2. Honey is non-compliant if it does not comply to the prescribed legislation per physico-chemical parameter and confined to the definition of honey as defined in the APS Act (DAFF, 2000). The non-compliance of the various parameters tested could be due to variations in pre- and post-production processes or adulteration (Al-Farsi, et al., 2018; Oroian, Olariu, & Ropciuc, 2018). The local samples had the highest compliance rate for HMF content (97.9%). The imported samples had the highest compliance rate for moisture and ash content (99%).

Specific rotation is an additional quality parameter the IHC proposes to distinguish between blossom and honeydew honeys and no limits have been set internationally (Bogdanov, et al., 1999; DAFF, 2000). Specific rotation of honey is the result of carbohydrates ability to rotate linear polarised light. Negative specific rotation of nectar honeys results from the predominance of fructose, while honeydew honeys have positive values due to the lower content of fructose and higher contents of di- and oligosaccharides that have positive specific rotation. In addition, the differences in honey specific rotation resulting from different carbohydrate profiles can also contribute to nectar honey characterisation. Although standards were published locally they not yet enforced. It was found that both the local samples (21%) and imported samples (30%) have a low compliance rate to the standards set in the APS Act.

Table 3.2: Compliance of physico-chemical parameters of locally produced and imported honey in South Africa according to requirements of the Agricultural Products Standards (APS) Act, 1990 (Act No 119 of 1990)

Physico-chemical parameter	Standards and requirements for compliance	Compliance of local honey (%)	Compliance of imported honey (%)
Sucrose (%)	* ≤5	87.3% n=758 Average=2.22 Std Dev=2.95	93.5% n=61 Average=1.27 Std Dev=1.71
Reducing sugars (%) (Sum of fructose, glucose and maltose)	* ≥65 (flowers) * ≥60 (honeydew)	85.7% n=784 Average=71.7 Std Dev=6.96	86.0% n=62 Average=72.0 Std Dev=6.57
Ratio of fructose:glucose	*Shall not be less than 1.0:1	80.4% n=784 Average=1.12 Std Dev=0.156	85.7% n=62 Average=1.16 Std Dev=0.206
Total acid (meq/kg)	* ≤40	96.1% n=729 Average=23.7 Std Dev=9.04	93.7% n=59 Average=24.7 Std Dev=13.26
Moisture (%)	* ≤20	97.3% n=780 Average=16.8 Std Dev=1.72	98.6% n=66 Average=17.1 Std Dev=1.22
Lund (cm^3)	* ≥0.6	92.3% n=767 Average=1.78 Std Dev=0.955	90.4% n=69 Average=2.20 Std Dev=1.34
Hydroxy-methyl-furfural (mg HMF/kg)	* ≤40	97.9% n=746 Average=10.1 Std Dev=12.4	94.0% n=63 Average=15.7 Std Dev=21.4
Specific rotation (°)	* Direct and immediate specific rotation- of an aqueous solution containing 26 g of floral honey in a total volume of 100 ml, shall not be less laevorotatory than -10 degrees at 20 °C.	20.8% n=764 Average=-13.2 Std Dev=6.47	29.9% n=64 Average=-14.2 Std Dev=7.97
Ash (%)	*≤0.6	97.1% n=767 Average=0.228 Std Dev=0.162	98.6% n=65 Average=0.163 Std Dev=0.146

* Agricultural Product Standards Act, 1990 (No 119 of 1990)

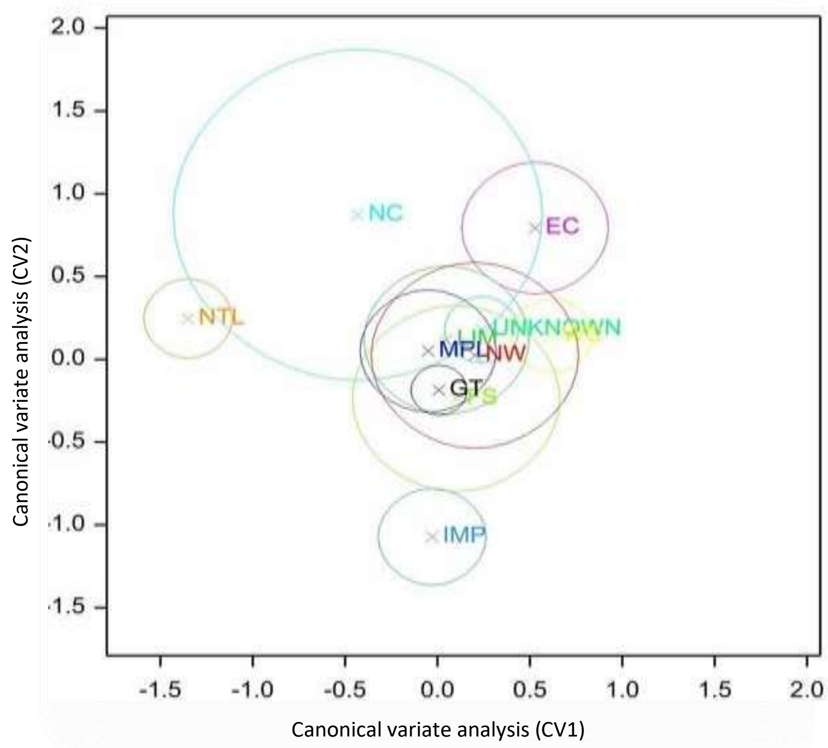

Figure 3.2: CVA analyses showing plot of mean scores for nine (9) physico-chemical parameters

In South Africa, honey is produced from an estimated 30 000 diverse plant species (Guelpa, Marini, du Plessis, Slabbert, & Manley, 2017). In addition, the species of honey bees (i.e. European vs African) also contribute to variation (da Silva, Gaunche, Gonzaga, Costa, & Fett, 2016). Two sub bee species, namely *A. m. scutellata* and *A. m. capensis* are responsible for honey production in South Africa (Gous, Willows-Munro, Eardley, & Swanevelder, 2017). *A. mellifera scutella* occurs in the northern parts of the western and southern cape region whereas *A. mellifera capensis* is present in the western and southern Cape regions (Masehela, 2017). Adulteration entails directly adding commercial syrup, cane and other sugars to the honey (Olawode, Tandlich, & Cambray, 2018). It can also be done indirectly by feeding bees with a concentrated sucrose solution within the beehives, which encourages bees to collect pollen instead of nectar (Crane, 1990).

For the comparison in quality of local and imported samples, the first two canonical variates (CV1 and CV2) accounted for 71.9% of the total variation among groups. A plot of the first two canonical mean scores per region using 95% confidence indicated that the imported samples (IMP) differed significantly from all other samples and contrasted mostly with the NTL, NC and EC regions (Figure 3.2). Furthermore, the regions FS and GT were most similar, as were MP, NW, LIM and UNKNOWN (UNK). The scores found for each of the CVs were then correlated with the original parameters to find those that are the most important in discriminating between the groups. The parameters mostly distinguishing between groups were the logarithms of total acid (r=0.81), sucrose (r=0.65), and to a lesser extent the square root of ash (r=0.55) and specific rotation (r=0.54).

The difference in quality between the locally produced and imported honey could be due to the fact that all honey that is imported into South Africa has to be irradiated as specified by the Agricultural Pests Act, 1983 (Act No 36 of 1983) (DALRRD, 1983). Irradiation significantly decreases the moisture, vitamin E contents and HMF level (Hussein, Yusoff, Makpol, & Mohd Yusof, 2014). The variation in the sucrose values, which were 0-32.40% and 0-8.84% for the local and imported honey, respectively, is possibly due to the large variety of plants in South Africa. The sucrose level in honey is used to indicate its degree of maturity, botanical origin and is the most common indicator for adulteration (Soares, Amaral, Oliviera & Mafra, 2017). It has been reported that early harvested honey has a higher sucrose content as the invertase has not broken it down to fructose and glucose yet (Chua & Adnan, 2014).

The interrelationship between the individual sugars could possibly explain the high level of variability observed in the F/G ratio, which varied from 0.536 to 2.27 and 0.787 to 1.69 in the local and imported honey, respectively (Olawode, Tandlich, & Cambray, 2018). The F/G ratio is an indication of the maturity of honey as well as honey blends (Salazar, de Freitas, da Luz, Bersch, & dos Santos Salazar, 2017). The lower the F/G ratio, the higher the tendency of the honey to crystallise (Halouzka, Tarkowski, & Cavar Zeljkovic, 2016). The decomposition of sucrose to glucose and fructose influences its invert sugars, F/G ratio and specific rotation indicating an interrelationship between the individual sugars.

The acidity level varied for the local and imported honey between 3.870-88.99 meq/kg and 5.120 to 88.25 meq/kg, respectively. The total acid content of honey, which prevents deterioration, is used to determine whether fermentation of fructose has taken place, which subsequently leads to an increase in HMF (da Silva, Gaunche, Gonzaga, Costa, & Fett, 2016). Variation in the acidity level in the sampled honey may be due to variation in harvest season, floral sources, geographical origin, acids produced by bacteria and the minerals present (Silva, Sousa, & Taveira, 2017).

The ash content is used to indicate floral origin and the purity of honey (Salazar, de Freitas, da Luz, Bersch, & dos Santos Salazar, 2017). Factors influencing the ash content include geographic origin, processing practices as well as pollution (da Silva, Gaunche, Gonzaga, Costa, & Fett, 2016). These may have contributed to the variability of the ash in local honey (0.228-0.001%) and in imported honey (0.163-0.002%) observed amongst the honey samples.

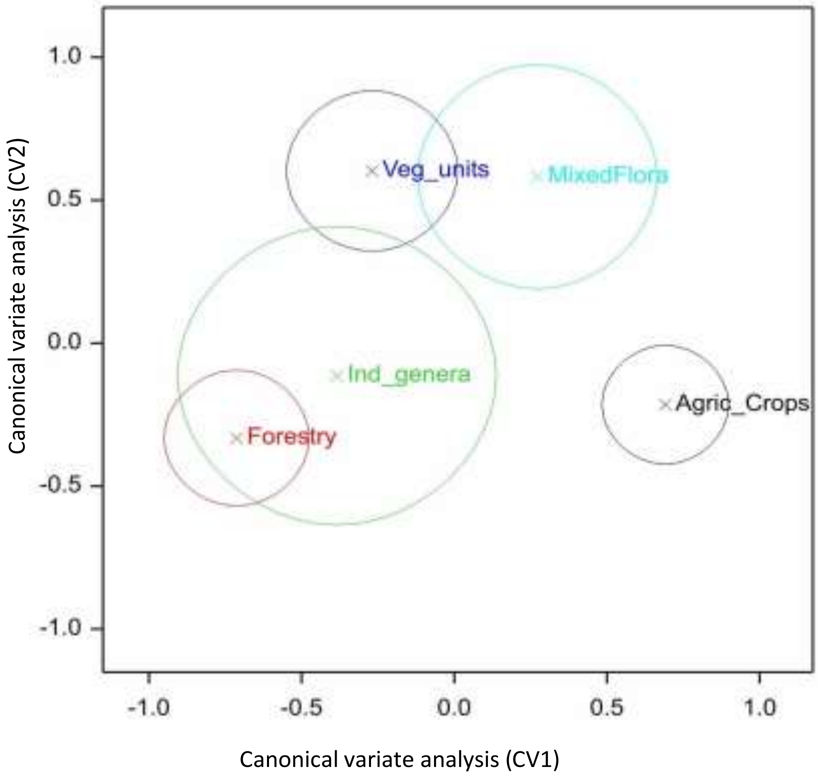

Figure 3.3: CVA analyses showing plot of mean scores for five forage types Agric_Crops – Agricultural Crops; Ind_genera –Indigenous genera; Veg_units – Vegetation units

South Africa's rich diversity of plant species provides enough bee forage to produce various types of honey with distinct characteristics (e.g., monofloral and polyfloral) (Guelpa, Marini, du Plessis, Slabbert, & Manley, 2017; Cengiz, Tosun, & Topal, 2018). Since mislabelling of origin is adulteration, it is important to classify and identify the origin of honey in relation to its physico-chemical properties (Chua & Adnan, 2014). Consequently, the quality groupings of different forage types were determined by a canonical variate analysis (CVA) to explore if any similarities between forage types exists. For the comparison in quality within local samples from different botanical origins, the first two canonical variates (CV1 and CV2) accounted for 92.1% of the total variation among groups.

A plot of the first two canonical mean scores per region using 95% confidence regions indicated that the agricultural crops differed significantly from all other forage types (Figure 3.3). Furthermore, mixed flora and vegetation differed significantly from forestry but not from each other. The parameters that mostly distinguished between forage types were Lund (r=+0.64), HMF (r=0.63) and, as with the previous CVA (Figure 1), ash content (r=-0.86) and to a lesser extent the logarithm of total acid (r=0.58). Both the Lund and HMF physico-chemical parameters are influenced by the sugar profile and these can be changed by adulteration. In addition, the HMF is also influenced by various factors including the presence of organic acids, pH, moisture content and storage time (White, 1975; da Silva, Gaunche, Gonzaga, Costa, & Fett, 2016). The quality of honey is a multifactorial parameter determined various factors including beespecies and the botanical- and geographical production environment (da Silva, Gauche, Gonzaga, Costa, & Fett, 2016; Soares, Amaral, Oliveira, & Mafra, 2017).

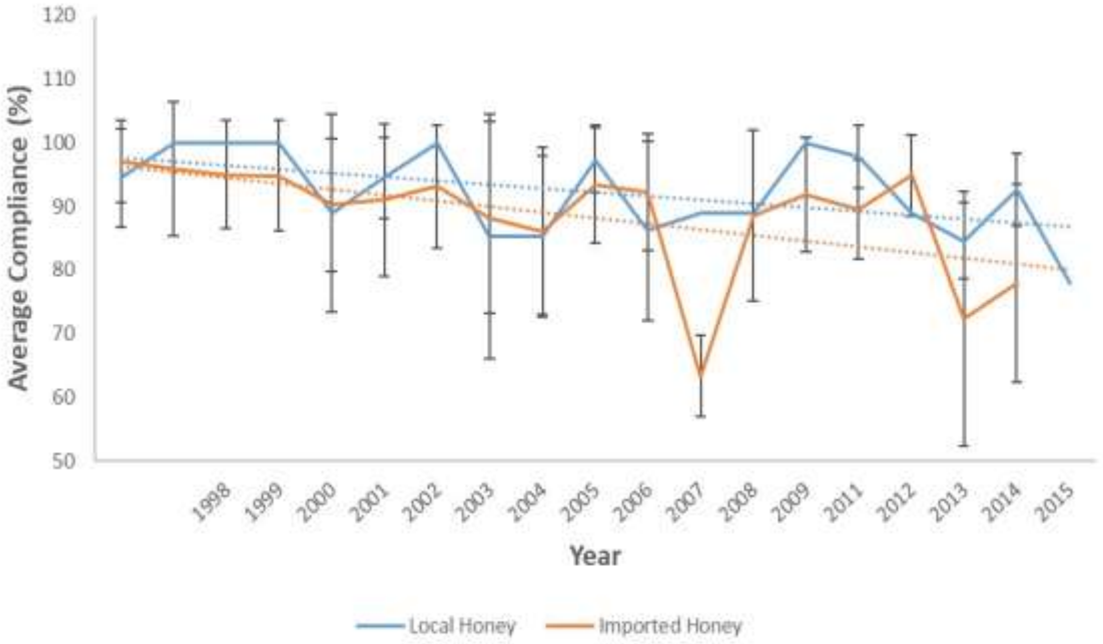

Figure 3.4: Quality of locally produced and imported honey in South Africa from 1998 to 2017

Global honey production pressures such as the declining honeybee population may lead to increased incidences of adulteration (da Silva, Gaunche, Gonzaga, Costa, & Fett, 2016). Even though most of the honey sold on the South African market is in accordance with regulations specified by the APS Act, a notable decrease in the quality of the South African honey samples was observed between 2008 to 2011 and 2014 to 2017 respectively (Figure 3.4). Between 2008-2009, South Africa experienced a severe drought, which may have influenced honey production. During this period, honey imported from other countries increased and more blended honey was sold on the market.

A limitation to the study is that samples were grouped together according to the address on the label of the containers. Mislabelling of geographical and botanical origin is considered an indirect form of adulteration (Codex Alimentarius, 2001; European Commission, 2002). This type of adulteration is growing worldwide (Soares, Amaral, Oliveira, & Mafra, 2017).

3.5 Conclusions and Recommendations

According to this study, most of the local and imported honey samples analysed complied to the APS Act and is generally of acceptable quality. This is mainly due to the enforcement of the regulatory framework of South Africa, which ensures that honey available on the market is of acceptable quality.

Authentication does, however, not just entail the evaluation of the quality of honey products but is also linked to consumer and market demands as it protects the consumer against adulterated, falsely labelled and fake products on the market.

Regulatory frameworks need to be assessed and revised more frequently and implemented more strictly. In addition, the interaction between consumers, producers and legislator's regional norms/quality criteria should be established for different types of honey as this is not specified in the South African legislation. Proper characterization of honey can increase the consumer's awareness of honey on the market. More rigorous methodology to determine quality should be developed and encouraged to ensure the integrity of this sought-after product on the market.

Significant differences in some of the physico-chemical parameters were observed between honey from different floral sources, with honey from agricultural crops accounting the biggest variation. Research on the floral origin and different agricultural crops should be encouraged to investigate the possibility of labelling honey from a specific floral source as a product of origin. This could be an economic incentive for farmers to continually produce a high-quality product. An example of this is Manuka honey, New Zealand's most popular honey.

Although DAFF currently only uses standard analytical tests as recommended by the IHC to determine if certain physico-chemical parameters are within set boundaries of the APS Act, new analytical techniques with higher accuracy should be implemented in future. Methods that are increasingly being used internationally include nuclear magnetic resonance (NMR), Infrared, chemometric-intergrated techniques, biosensor and carbon isotope analyses. The latter can be used to determine from which carbon source plants (C3/4) honey was produced and to verify authenticity.

3.6 Credit Statements

Tersia de Beer: Conceptualization, Methodology, Software, Formal analysis, Resources, Investigation, Writing & editing.

Margot Otto: Writing & editing - original draft.

Beulah Pretorius: Supervision, Conceptualization, Writing - review & editing.

Hettie Schönfeldt: Project administration, Supervision, Conceptualization, Funding acquisition.

3.7 Acknowledgments

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3.8 Conflict of interest

The authors declare no conflict of interest.

3.9 References

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CHAPTER 4: HOW DO SOUTH AFRICAN HONEY MEASURE AGAINST INTERNATIONAL AND LOCAL QUALITY STANDARDS

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4.1 Abstract

The global decline in production and a continual increase in the demand for honey makes it prone to adulteration and honey is considered as one of the seven foods most likely to be adulterated. Honeys from different botanical and geographical origins have specific compositional fingerprint. Hence, honeys (n=14) from C_3, C_4 and crassulacean acid metabolism (CAM) plants were tested in this study to monitor their origin and quality. Selected physico-chemical parameters, melissopalynological and stable carbon isotope analyses were performed to determine specific compositional profiles. Various factors contributed to the compositional differences in maturity, purity, degree of deterioration and adulterants. Melissopalynology could confirm botanical origin, but the geographical origin could not be confirmed neither by melissopalynology, nor by stable carbon isotope analyses. Sugar adulteration could not be confirmed by stable carbon isotope analyses.

Keywords: *C_3, C_4, CAM plants, compositional profile, isotope analysis, melissopalynology*

4.2 Introduction

Honey is a food product with a high commercial value naturally produced by honeybees from nectar and honeydew (secretion by plant-sucking insects) (Codex Alimentarius, 2001; Guelpa, Marini, du Plessis, Slabbert, & Manley, 2017). South Africa's unique floral biodiversity provides an abundant variety of nectar and pollen producing plants for honey production. Monofloral honey is produced from a single flower species from a specific botanical or geographical origin and is considered to have a higher commercial value than polyfloral honey. In addition, it is prone to mislabelling and fraudulent admixing with inexpensive honeys (Guelpa, Marini, du Plessis, Slabbert, & Manley, 2017). For example, in China and Venezuela the blending of acacia honey with rape honey, (a less expensive honey) is common practice (Fakhlaei, et al., 2020). This example highlights why it is necessary to authenticate honey available on the South African market. This may entail verification of the botanical and geographical origins as declared on labels, as well as detecting the potential adulteration of honey with various sugars and sugar syrups (Fakhlaei, et al., 2020). Sugar syrup can be classified as a honey substitute according to the APS Act. *"Honey substitutes means foodstuff consisting of food substance or a mixture of substances that have been made to resemble honey".* Sugar Cane honey is produced from sugar cane sap which are secreted from cane straws (DALRRD, 2000). It is important to note that produce resulting from feeding bees with sugar syrup, is not defined as honey, but it is defined as indirect adulteration of honey (DALRRD, 2000; Fakhlaei, et al., 2020).

The distinct characteristics of honey types are influenced by the effect of environmental factors (floral and geographic origins) and processing (e.g., harvesting and storage) variables during production. However, the chemical composition and sensory characteristics of honey depend on the botanical origin. Consequently, the aim of food authentication is to select and analyse specific quality parameters where alterations during processing can easily be detected and accurately measured and quantified (Fakhlaei, et al., 2020). The quality of honey is determined by its chemical composition, and sensory, physical and microbiological characteristics (Soares, Amaral, Oliveira, & Mafra, 2017). In South Africa, the authenticity of honey is currently verified through testing of selected physico-chemical parameters, such as sugars (sucrose, fructose, glucose and maltose, fructose/glucose ratio), moisture, ash, total acidity, Lund, specific rotation and hydroxyl-methyl-furfural (HMF). A honey must comply within a range of regulated values as specified by the Agricultural Product Standards (APS) Act (Act No 119 of 1990) (DALRRD, 2000).

Complementary to the physico-chemical analysis, melissopalynological, as well as stable isotope analysis, were included to verify authenticity. Melissopalynology is recognised worldwide as being an official method for identifying botanical origin, while stable isotope analysis is the official method to detect adulteration with C_4 sugars. The principle of the stable isotope analysis is based on the fact that C_3, C_4 and CAM plants have distinct carbon isotope ratios due to different photosynthetic pathways (Fakhlaei, et al., 2020). Melissopalynology is recognised worldwide as the reference method to identify the floral sources, the potential that adulteration has occurred and the determination of local or foreign origin of a sample (Seraglio, et al., 2019).

Different approaches have been used to characterise honeys of specific botanical and geographical origins, as there is no one universal approach that unequivocally discriminates between honey types. So multiple approaches, that complement each other would be more reliable to characterise honeys of different botanical and geographical origins. The aim of this study was to assess honey samples originating from plants with different photosynthetic pathways (C_3, C_4 and CAM) by using internationally validated methods including the testing of selected physico-chemical parameters (pH-value, total acidity, moisture, refractive index, total soluble solids, ash, Lund's reaction and sugars), melissopalynological, as well as stable carbon isotope analyses for comparative purposes to 1) verify authenticity of origin (botanical and geographical) and 2) potentials of adulteration, possibility of [by means of] blending of honey and honeys of different quality.

4.3 Materials and Methods

4.3.1 Sampling

A total of 14 samples which included sugar cane honey (SC) (n=1) and a honey sample labelled as honey sugar syrup (SS) (n=1) were analysed. The honey samples were obtained from some of the most commonly utilised floral sources in South Africa; namely *Eucalyptus* (n=5, E1-E5), sunflower (n=5, S1-S5) and aloe (n=2, A1-A2). The samples were obtained directly from beekeepers from five of the nine South African provinces namely: North West, KwaZulu-Natal, Gauteng, Mpumalanga and Limpopo. In addition, an imported sample of Australian *Eucalyptus* honey was included for comparative purposes. In addition, the sugar cane honey sample originated from KwaZulu-Natal and the honey sugar syrup sample was from Gauteng. (Table 4.1). All samples were stored in airtight containers and kept at room temperature required for analysis.

4.3.2 Evaluation of the physico-chemical properties

4.3.2.1 pH-value, total acidity, moisture, refractive index, total soluble solids, ash and Lund's reaction

All samples were analysed in duplicate using methods recommended by the Official Methods of Analysis (AOAC), the International Honey Commission (IHC) and the Codex Alimentarius Honey Standards (AOAC, 1995; Bogdanov, et al., 1999; Codex Alimentarius, 2001). All samples were analysed by the National Analytical Services, Directorate Food Safety and Quality Assurance, Department of Agriculture, Land Reform and Rural Development in Pretoria as previously described by de Beer, Otto, Pretorius and Schönfeldt (2021).

4.3.2.2 Sugars

Sucrose, fructose, glucose and maltose contents were determined based on the AOAC 982.14 method using High Performance Liquid chromatography (HPLC) (AOAC, 2011).

4.3.2.3 Melissopalynology

The melissopalynology analysis were done at Prosper Bande (Evolutionary Studies Institute), University of the Witwatersrand, Johannesburg using a standard method for pollen processing in melissopalynology. The pollen was identified by microscopic analysis (Lieux, 1980; Jones, 2014). The standard method for pollen processing in melissopalynology is described (Lieux, 1980; Jones, 2014).

Honey samples respectively syrups (c. 10g) were heated with aqua dest in the palynology laboratory at Evolutionary Studies Institute (ESI). Acetolysis (9:1 ratio of acetic anhydride to sulfuric acid) is applied to dissolve proteins, e.g., cytoplasm and the inner layer of the pollen wall, the intine. Since pollen, identification is based on the investigation of the sculpture and structure of the exine (Halbritter, et al., 2018). This process allows easier staining, photographing and identification (Jones, 2014). The residue was mounted onto glass slides using glycerine jelly, microscopic slides are stored at ESI. For the analysis, an Olympus light microscope (CX31 respectively CX23, magnification x4730, oil immersion) including Dinolite 5MP camera was used (image analysis software DinoCapture 2.0, e.g., for stacking, photo plates). Minimally 300 pollen grains have to be analysed for an estimation of the relative frequencies of pollen types, but a pollen count of 500-1000 pollen grains is needed for the determination of relative frequencies and to detect rare taxa (Behm, von der Ohe, & Henrich, 1996).

Table 4.1: Honey samples (Fresh Weight) obtained from various provinces in South Africa including a sugar cane and a sugar syrup sample, as well as an imported honey sample from Australia

Honey Identification number	Province	Location	Harvest period
E1	Gauteng	Bronkhorstspruit	June 2019
E2	Mpumalanga	Piet Retief	June 2019
E3	Mpumalanga	Standerton	June 2019
E4	Mpumalanga	Sabie	May 2019
E5	Imported (Blue Mountains)	Australia	2019
S1	Mpumalanga	Middelburg -Stofberg	March 2019
S2	Mpumalanga	Standerton	February-March 2019
S3	North West	Lichtenburg	February 2019
S4	North West	Ventersdorp	March 2019
S5	Limpopo	Roedtan	March 2019
SC	Kwa-Zulu Natal	Merrivale	2019
SS	Gauteng	Randfontein	2018
A1	Gauteng	Wallmansthal	June 2019
A2	Limpopo	Modimolle	August 2018

4.3.2.4 Stable carbon isotope analysis

The stable carbon isotope analyses were done at the Stable Isotope Laboratory at the University of Pretoria. It is important to note that at this stage of the research, a protein extraction as described by the AOAC 988.12 was not done, as only bulk samples of each honey were subjected to isotopic analyses, together with the various sugars (AOAC, 2005).

All honey and sugar samples were tested in triplicate. A subsample of between 3.1 ± 0.7 mg of the honey samples and 0.5 ± 0.1 mg of the syrup or sugars samples were weighed into clearly marked tin capsules, which were pre-cleaned with toluene. Two in-house laboratory standards with known $\delta^{13}C$ ratios, Merck Gel ($\delta^{13}C$=-20.26‰) and DL-Valine ($\delta^{13}C$=-10.57‰), were repeatedly analysed together with the samples. The in-house running standards were calibrated using the following international calibration standards, NBS 22 (oil), IAEA-CH-3 (cellulose), IAEA-CH-6 (sucrose), and IAEA-CH-7 (polyethylene foil). These calibration standards are supplied by the United States Geological Service (USGS) and the International Atomic Energy Agency (IAEA). The mass of running standards and sugars was varied as a means of accounting for any potential sample size effect.

Samples were combusted at 1 020°C using a Flash 1112 Series Elemental Analyzer linked via a Conflo IV interface to a Delta V Plus stable light isotope ratio mass spectrometer (IRMS), housed at the University of Pretoria Stable Isotope Laboratory. All the equipment was supplied by Thermo Scientific, Bremen, Germany.

The $\delta^{13}C$ values obtained for the in-house Merck Gel and DL-Valine standards (n=10 of each) were used to calibrate the sample results and provide the instrumental precision for the run, which was <0.08‰ for carbon. There was no sample size effect on the $\delta^{13}C$ values obtained during the isotopic analyses, nor any significant differences between the results for the triplicates. All stable carbon isotope values are presented in delta (δ) notation on a per mille (‰) scale, relative to the Vienna PeeDee belemnite (VPDB) international standard, using the standard equation:

$$\delta X\ (‰) = (R_{sample}/R_{standard}) - 1$$

Where X = ^{13}C and R represents $^{13}C/^{12}C$ (Coplen, 2011).

Note that $\delta^{15}N$ ratios were measured for each sample, but peak areas were often too small or there was insufficient nitrogen to permit measurement. When nitrogen isotope ratios were obtained, these values varied to such a degree between triplicates and showed a strong sample size effect. Therefore, nitrogen isotope results obtained for the bulk samples were not considered for this publication. As previously mentioned, no protein extraction and subsequent carbon isotope analysis of the extracted protein was done.

4.3.2.5 Statistical analysis

The data for the physico-chemical parameters and stable carbon isotope ratios of samples analysed was summarised and imported into GenStat (VSN International, 2017) for evaluation (VSN International, 2017). The dataset (Tables 4.2 and 4.3) was used to perform a Principle Component Analysis (PCA) to determine the quality groupings of the control and honey samples originating from plants with different photosynthetic pathways (C_3, C_4 and CAM) and different geographic locations (Krzanowski, 1988). A qualitative pollen analysis was done and its data is presented in Table 4.4. Frequency classes were determined as predominant pollen types (>45%), secondary pollen types (>16-44%), important minor pollen types (>3-15%) and minor pollen types (<3%). Honey samples containing more than 45% of a single type of pollen were considered as monofloral honey (Louveaux, Maurizio, & Vorwohl, 1978). A qualitative stable carbon isotope analysis was done on the bulk honey to determine isotopic ratios.

4.4 Results and Discussion

The physico-chemical parameters to verify the quality of the honey and to detect possible adulterants in the honey samples are summarised in Tables 4.2 and 4.3. All the honey samples complied with the prescribed range of sucrose (<5%) indicated in the APS Act, Codex Standards for honey (Codex) and European Directive (EU) (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002). The Australian *Eucalyptus* honey (E5) had the highest sucrose content of 1.2% of all nectar honeys, while the sugar syrup (SS) had the highest sucrose content of 15%, as expected.

The variation in the sucrose content is due to the fact that, not all the sucrose has been hydrolysed by the invertase enzyme into simple monosaccharides (fructose and glucose) (Machado De-Melo, Almeida-Muradian, Sancho, & Pascual-Maté, 2018). Low sucrose contents indicate that sucrose is completely converted into glucose and fructose during the ripening process (Al-Farsi, et al., 2018). A high sucrose content (>8%), is related to its botanical origin, honey immaturity or potential artificial feeding of bees with sugars such as a concentrated sucrose solution or the direct addition of commercial sugar syrup to honey. The most commonly used adulterants for honey are sucrose, corn syrup, molasses and crushed banana (Fakhlaei, et al., 2020). Some honeys possess a naturally high sucrose content, such as *Lavender (Lavandula spp)* and *Borago* (*Borago officinalis*). The international standards of Codex and the EU Directive prescribed a sucrose content of less than <15%, honey from the *Robinia, Medicago, Banksia, Hedysarum, Eucalyptus, Eucryphia* spp, and *Citrus* spp sucrose content should not be more than 10% (Codex Alimentarius, 2001; European Commission, 2002).

The set limit of the reducing sugars (sum of the fructose, glucose and maltose) must be $\geq$60 g/100 g (DALRRD, 2000). In this study, the prominent sugars in the nectar honeys were fructose and glucose, with sucrose less prominent and in minor concentration (<2%). All three of these sugars are indicative of the maturity of honey (Seraglio, et al., 2019). Most of the honey sampled complied with the nationally and internationally prescribed set limits (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002). However, E5 honey (55.4%) was lower than the set limit, as well as the SC (53.4%) and SS (58.9%). The lower reducing sugar content in honey can be due to the type of flower (nectar), ripeness, storage, processing (weather exposure, heating processes, packaging), the types of enzymes deposited by the honeybees, as well as to adulteration with other products (Aljohar, et al., 2018; Machado De-Melo, Almeida-Muradian, Sancho, & Pascual-Maté, 2018).

The enzyme invertase plays a role in the concentration of glucose and fructose in honey, because invertase breaks down sucrose into fructose and glucose (Pavlova, Stamatovska, Kalevska, Dimov, & Nakov, 2018). Glucose depends on the source of nectar, while fructose is broken down to HMF naturally via acid catalysation during storage (Seraglio, et al., 2019).

No maltose was found in any of the samples (Table 4.2). In a study done by Poyrazoglu, et al., (2012), on Turkish honey, maltose was also not detected in some of the honey samples derived from cotton, sunflower or multi-floral origin and in other honeys in an inconsistent manner (Poyrazoglu, Haroun, Konar, Hospolat, & Artik, 2012). This is an indication that maltose does not originate in the nectar but arise from the transglycosylation activity of α- and β- glucosidases enzymes contributed by the honeybees during the honey process (Low, Va Vong, & Sporns, 1986; Low, Nelson, & Sporns, 1988). The fructose/glucose ratio (F/G) prescribed in the APS Act, should not be less than 1:1. The F/G ratio of all honeys (1.3-1.4), SC (1.3) and SS (1.1) was compliant to the set limit (DALRRD, 2000).

The pH-value ranged from 3.35-4.39 for all the honey samples (Tables 4.2 and 4.3, indicating the acidic nature of honey and were within the typical acceptable range of 3.4-6.1 (Osuagwu, Oyerinde, Onipede, & Ombugadu, 2020). The mean pH-values for the *Eucalyptus,* sunflower and *Aloe* honeys were 3.86 ± 0.417, 3.51 ± 0.146 and 3.6 ± 0.021, respectively. Furthermore, the pH-value of SC was 4.11 and SS 3.35. There is no international legislation that specifies acceptable limits of pH-values, although, pH plays an important role in the stability, texture and shelf-life of honey (Seraglio, et al., 2019). In addition, honey is acidic regardless of geographical origin (Osuagwu, Oyerinde, Onipede, & Ombugadu, 2020).

Table 4.2: Combination of the sugar, pH-value and total acidity results for the Eucalyptus, Sunflower, Aloe honey samples, the Sugar cane and Sugar syrup samples

Honey Identification number	Sucrose (%)	Fructose (%)	Glucose (%)	Maltose (%)	Fructose + glucose + maltose (%)	F/G	Mean pH ± SD	Mean Free Acid (meq/kg) ± SD	Mean Lactone (meq/kg) ± SD	Mean Total Acid (meq/kg) ± SD	Mean Lactone / free acid ratio (meq/kg)
E1	<0.1	41.1	27.0	0.0	68.1	1.52	3.36 ± 0.00	10.75 ± 0.71	4.50 ± 0.35	15.25 ± 0.35	0.419
E2	<0.1	36.8	27.4	0.0	64.2	1.34	4.39 ± 0.01	12.00 ± 0.00	2.62 ± 0.53	14.62 ± 0.53	0.219
E3	<0.1	38.0	27.5	0.0	65.5	1.38	3.64 ± 0.00	14.25 ± 0.00	5.50 ± 0.00	19.75 ± 0.00	0.386
E4	<0.1	40.2	29.1	0.0	69.3	1.38	4.17 ± 0.00	20.87 ± 0.18	8.12 ± 0.18	29.00 ± 0.35	0.389
E5	1.2	32.9	22.5	0.0	55.4	1.46	3.72 ± 0.00	11.50 ± 0.35	3.50 ± 0.00	15.00 ± 0.35	0.304
S1	<0.1	53.2	33.2	0.0	86.4	1.60	3.40 ± 0.00	12.75 ± 0.00	6.37 ± 0.18	19.12 ± 0.18	0.500
S2	<0.1	41.9	30.2	0.0	72.1	1.39	3.53 ± 0.01	11.50 ± 0.00	7.50 ± 0.00	19.00 ± 0.00	0.652
S3	<0.1	38.8	33.6	0.0	72.4	1.15	3.37 ± 0.01	14.50 ± 0.35	8.00 ± 0.35	22.50 ± 0.71	0.552
S4	<0.1	41.9	29.5	0.0	71.4	1.42	3.74 ± 0.00	17.50 ± 0.35	5.25 ± 0.00	22.75 ± 0.35	0.300
S5	<0.1	39.2	32.0	0.0	71.2	1.23	3.53 ± 0.01	12.50 ± 0.00	4.75 ± 0.35	17.25 ± 0.35	0.380
SC	<0.1	30.2	23.2	0.0	53.4	1.30	4.11 ± 0.00	31.87 ± 0.18	6.00 ± 0.71	37.87 ± 0.88	0.188
SS	15.0	31.0	27.9	0.0	58.9	1.11	3.35 ± 0.01	17.37 ± 0.18	1.75 ±0.35	19.12 ± 0.18	0.101
A1	<0.1	41.3	32.0	0.0	73.3	1.29	3.58 ± 0.00	16.25 ± 0.35	7.75 ± 0.00	24.00 ± 0.35	0.477
A2	<0.1	40.9	32.7	0.0	73.6	1.25	3.61 ± 0.00	10.50 ± 0.35	4.75 ± 0.71	15.25 ± 035.	0.452

Eucalyptus=E1; *Eucalyptus*=E2; *Eucalyptus*=E3; *Eucalyptus*=E4; *Eucalyptus*=E5; Sunflower=S1; Sunflower=S2; Sunflower=S3; Sunflower=S4; Sunflower=S5; Sugar Cane=SC; Sugar Syrup=SS; Aloe=A1; Aloe=A2; Fresh Weight

The acidity of honey is linked to the presence of organic acids, which is closely connected to, and balanced with the content of lactones and can be evaluated as free acid, lactonic acid and total acid (sum of free acid and lactone). The set limit of the total acidity as prescribed in the APS Act, 1990 is less than 40 meq/kg. (DALRRD, 2000). The total acidity (sum of free acid and lactone) of the samples varied between 14.62-37.87 meq/kg, whereas the free acid varied between 10.50-31.87 meq/kg and the lactone between 1.75-8.12 meq/kg. All the total acidity values were in acceptable limits as prescribed indicating the freshness of these honeys (DALRRD, 2000; Osuagwu, Oyerinde, Onipede, & Ombugadu, 2020). The lactone values of the studied honeys were higher than the international recommended standard limit of 3.23 meq/kg except for E2 (2.64 meq/kg) and SS (1.75 meq/kg) (Codex Alimentarius, 2001). Factors contributing to the variation in the acidity level include the harvest seasons, different floral sources, geographical origin, acids produced by bacteria and the minerals that are present (Al-Farsi, et al., 2018; Seraglio, et al., 2019).

The variable proportion of lactone is expressed as the ratio of lactone to free acid. The lactonic/free acidity ratio (L/FA) ranged between 0.22-0.65 for the different honey samples, whilst the SC and SS, L/FA ratio values were 0.19 and 0.10, respectively. According to White (1975), regarding American honeys, the average L/FA ratio values for all floral honeys is 0.36 and for honeydew 0.13. This ratio variation is affected by the botanical and geographical origins. This author suggested that there is a possible relationship between the L/FA ratio and the pH of the sample. The lower the pH value, the greater the concentration of lactones and thus the higher the L/FA ratio (White, 1975). The honey lactones exhibited a wide variation hence, no relationship could be found in our study.

Moisture is the second most important component in honey and has a set limit of less than 20% (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002). Normally, honey moisture content ranges between 13-25%, being optimal at 17%. Honeys with a low moisture content are difficult to handle and process, in contrast to honeys with a moisture content higher than 18%, which are prone to ferment (Machado De-Melo, Almeida-Muradian, Sancho, & Pascual-Maté, 2018). As a quality parameter, moisture content influences the fermentation rate, shelf life as well as the crystallisation rate of honey (Al-Farsi, et al., 2018).

The moisture content varied between 14.6% and 21.7% for all nectar honey, SC and SS samples (Table 4.3 and 4.4). The S1-S5 honey had the highest moisture content of 17.5-21.7%, followed by A1 and A2, (17.8-18.0%), SC (17.0%), E1-E5 (15.2-20%) and SS (14.6%). The two non-compliant S1 and S3 honey samples had a moisture content of 21.5% and 21.7%, respectively (Table 4.3). The SS fall within the set limit of 20% moisture content though they are adulterated products as prescribed by the APS Act (DALRRD, 2000). Factors influencing moisture include: harvesting period, environmental conditions (temperature and humidity), degree of maturity reached in the hive, moisture content of the original plant, industrial areas, handling and processing techniques and the fact that honey is also highly hygroscopic (Al-Farsi, et al., 2018). Hence, care should be taken when the honey is harvested. This may have contributed to the higher moisture values of S1 and S3, although both of them were harvested in the same year, under different environmental conditions and geographical regions (Warui, et al., 2019).

The °Brix values are directly related to the level of sugar in the honey, which represents the total soluble solids. The total solids are mainly sugars, with fructose and glucose accounting for 85% thereof (Osuagwu, Oyerinde, Onipede, & Ombugadu, 2020). The °Brix values were between 76.87% and 83.05% for the different samples and for the SC and SS the values were 81.27% and 83.56%, respectively. Honeys with higher sugar contents will exhibit higher °Brix values, as well as lower moisture contents (Al-Farsi, et al., 2018). In our study we found high °Brix values with a low moisture content. The water content and invert sugars content values were high, while the sucrose values were low, except E5 and SS, which had ~~a~~ low water content and invert sugars values and high sucrose level. In addition, the acid and mineral contents also contribute to the total soluble solids in honey (Al-Farsi, et al., 2018). The variation is due to the type of plants in the geographical area and also climatic conditions.

Bees forage over an area of more than 10 km^2 depending on the forage available. In monofloral honeys differences can be attributed the surrounding forage that changes with the geographical origin were the honey was harvested. However, if the geographical regions are close to each other the physico-chemical values will be the same (Sponsler & Johnson, 2015). All the honeys originated from five different regions in South Africa, namely North West (104 882 km^2), KwaZulu-Natal (94 361 km^2), Gauteng (18 178 km^2), Mpumalanga (76 495 km^2) and Limpopo (125 755 km^2), except for one (E5) that was sourced from Australia (List of South African provinces by area, 2021).

Currently, the APS Act, 1990 in South Africa does not require the completion of °Brix analysis to establish honey quality (DALRRD, 2000). Furthermore, according to the grading system of the United States of Department of Agriculture (USDA), honey with total soluble solids ≥81.4% is considered of a higher grade, while those falling between 80-81.3% are considered ~~a~~ lower grade (Osuagwu, Oyerinde, Onipede, & Ombugadu, 2020). According to the findings of this study (Table 4.3), only three (3) honeys can be considered high quality according to the USDA grading system, namely: E1, E2, E5, as well as the SS, whilst A1, A2, E3, S2 and S4 are considered lower grade, together with the SC. The other honeys, S1, S3, E4 and S5 all fall outside the grading system. Refractive Index is an optical characteristic in honey that varies between 1.5040 and 1.4815 according to the literature and increases when solid content is high and moisture content is low depending on the temperature (Machado De-Melo, Almeida-Muradian, Sancho, & Pascual-Maté, 2018). All the honeys in this study (Table 4.3) had a mean refractive index that falls within this range.

The maximum limit of ash content in honey shall not exceed 0.6% as prescribed in the APS Act (DALRRD, 2000). The ash content represents the mineral content of honey and is used to indicate floral origin and the purity of honey (Osuagwu, Oyerinde, Onipede, & Ombugadu, 2020). The ash content of the samples in this study falls in the range of 0.12-0.71% as indicated in Table 4.3. The following two samples did not comply with the set limit of less than 0.6%, E4 honey sample (0.71%) and the SC (0.69%). However, the differences observed from geographical origin may have been induced by the influences of the botanical origin of the honey. The variations of ash content can be attributed to location of harvesting (geographic origin), processing practices, as well as pollution. Some minerals cannot be detected in some types of honey collected from certain areas but are available in other regions due to the large dependence of mineral content on the soil and water compositions of such regions (Osuagwu, Oyerinde, Onipede, & Ombugadu, 2020).

The set limit for the Lund value in the APS Act, is a value of not less than 0.6 cm^3 (DALRRD, 2000). The Lund values range varied between 0.00-2.05 cm^3 (Table 4.3), with SC and SS, exhibiting values of 1.15 cm^3 and 0.00 cm^3, respectively. The Lund values of the nectar honeys and the SC honey fall into the acceptable limit of the prescripts indicated in the APS Act, except SS (DALRRD, 2000). The non-compliance of SS confirms that it is a syrup honey (adulterated). However, the pollen test indicated that it is a multiflora honey and the 15% sucrose value (Table 4.2) also indicated an adulterated honey. This quality criterion is used for differentiation between pure and adulterated honey. Respectively, the presence of albuminous cells (natural proteins) are an indication of the purity of honey, whereas their absence is an indication of adulteration (Marcucci, et al., 2019). The precipitate range of 0.6-3.0ml is indicative of the purity of honey (Salazar, Freitas, de Luz, & da Bersch, 2017).

As honeybees produce honey mainly from C_3 plants, honeys with a $^{13}C/^{12}C$ ratio (expressed as $\delta^{13}C$) values of -23.5‰ and lower are deemed pure. Furthermore, the difference between the $\delta^{13}C$ values of a honey sample and its protein fraction should not be more than 1‰. The C_3 plants $\delta^{13}C$ values range from of -23‰ to -28‰, C_4 plants $\delta^{13}C$ values range from of -9‰ to -15‰, and the CAM $\delta^{13}C$ values fall in the range of -11‰ to -13.5‰ (Chen, et al., 2019). The $\delta^{13}C$ values of the E1-E4 group ranged from -23.22‰ to -24.87‰, while E5 honey had a high value of -21.76‰. See Table 4.3. While S1-S5 $\delta^{13}C$ values ranged from -21.92‰ to -23.58‰ and A1 and A2 $\delta^{13}C$ values were -13.11‰ and -13.66‰, respectively. The difference in the $\delta^{13}C$ values of the honeys were due to the climatic conditions, feeding practices, as well as their different geographic origins (Chen, et al., 2019).

Table 4.3: Combination of the moisture, ˚Brix, refractive index, ash, Lund and isotope analysis for the Eucalyptus, Sunflower, Aloe honey samples, Sugar cane and Sugar syrup analysed

Honey Identification number	Mean Moisture (%) ± SD	Mean °Brix ± SD	Mean RI ± SD	Mean Ash (%) ± SD	Mean Lund (cm^{-3}) ± SD	Mean δ13C (‰) (triplicate) ± SD
E1	15.2 ± 0.00	83,05 ± 0.07	1.4988 ± 0,00020	0.154 ± 0.002	1.60 ± 0.28	-23.61 ± 0.447
E2	16.0 ± 0.00	82,26 ± 0.01	1.4967 ± 0,00004	0.441 ± 0.000	0.65 ± 0.49	-23.22 ± 0.703
E3	17.6 ± 0.00	80,71 ± 0.04	1.4926 ± 0,00012	0.221 ± 0.001	1.95 ± 0.07	-23.27 ± 0.218
E4	20.0 ± 0.00	78,34 ± 0.01	1.4864 ± 0,00002	0.713 ± 0.036	2,00 ± 0.00	-24.87 ± 0.558
E5	15.4 ± 0.00	82,74 ± 0.01	1.4979 ± 0,00003	0.181 ± 0.005	2.05 ± 0.07	-21.76 ± 0.200
S1	21.5 ± 0.14	76,87 ± 0.04	1.4826 ± 0,00008	0.173 ± 0.001	1,00 ± 0.00	-23.47 ± 0.561
S2	17.6 ± 0.00	80,74 ± 0.01	1.4927 ± 0,00001	0.204 ± 0.002	1.20 ± 1.13	-23.58 ± 0.083
S3	21.7 ± 0.14	76,91 ± 0.01	1.4821 ± 0,00021	0.119 ± 0.007	1.55 ± 0.64	-21.92 ± 0.378
S4	17.5 ± 0.14	80,75 ± 0.05	1.4927 ± 0,00013	0.310 ± 0.003	1.15 ± 0.07	-22.18 ± 0.087
S5	19.2 ±0 .28	79,16 ± 0.31	1.4885 ± 0,00081	0.234 ± 0.006	1.15 ± 0.07	-23.31 ± 0.107
SC	17.0 ± 0.00	81,27 ± 0.04	1.4941 ± 0,00012	0.692 ± 0.020	1.15 ± 0.07	-22.41 ± 0.463
SS	14.6 ± 0.00	83,56 ± 0.04	1.5001 ± 0,00012	0.504 ± 0.00	0 ± 0.00	-10.89 ± 0.114
A1	17.8 ± 0.00	80,49 ± 0.00	1.4920 ± 0,00001	0.306 ± 0.00	1.10 ± 0.00	-13.66 ± 0.438
A2	18.0 ± 0.00	80,33 ± 0.03	1.4916 ± 0,00008	0.252 ± 0.006	0.75 ± 0.07	-13.11 ± 0.548

Eucalyptus=E1; *Eucalyptus*=E2; *Eucalyptus*=E3; *Eucalyptus*=E4; *Eucalyptus*=E5; Sunflower=S1; Sunflower=S2; Sunflower=S3; Sunflower=S4; Sunflower=S5; Sugar Cane=SC; Sugar Syrup=SS; Aloe=A1; Aloe=A2; Fresh Weight

The geographical origin based on isotopic composition, could not be confirmed due to a lack of a database to verify geographical origin in South Africa. The δ^{13}C values of SC and SS samples were -22.41‰ and -10.89‰, respectively. The δ^{13}C value of SC (-22.41‰) can be attributed to the fact that this honey was identified as a nectar honey as the dominant pollen is from *Eucalyptus* and not a sugarcane honey (C$_4$-honey) as labelled. The δ^{13}C value of SS (-10.89‰) falls in the C$_4$ plants δ^{13}C values range of -9‰ to -15‰. In addition, it has a sucrose value of 15% and sucrose produced from cane sugar is derived from the C$_4$ photosynthesis cycle and is heavier in δ^{13}C. This indicates indirect adulteration of honey by overfeeding bees with C$_4$ sugar syrups (Eshete, 2019a). Other, literature indicates that δ^{13}C values for C$_4$-plants are -10‰ to -20‰ while C$_3$-plants δ^{13}C values are -22‰ to -33‰ (Zhou, Taylor, Salouros, & Prasad, 2018). Furthermore, the Lund value of 0.00 cm^3 is indicative of an adulterated product.

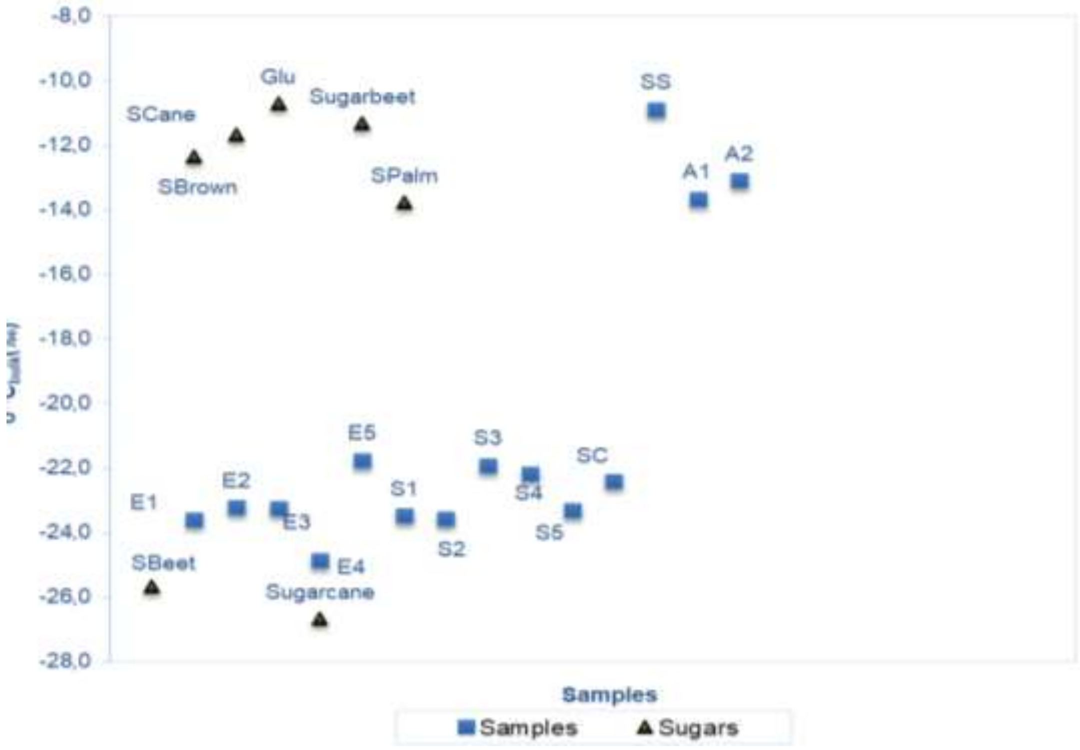

Figure 4.1: Mean (samples were run in triplicate) carbon isotope ratios obtained for the bulk honey samples and sugars Eucalyptus=E1; Eucalyptus=E2; Eucalyptus=E3; Eucalyptus=E4; Eucalyptus=E5; Sunflower=S1; Sunflower=S2; Sunflower=S3; Sunflower=S4; Sunflower=S5; Sugar Cane=SC; Sugar Syrup=SS; Aloe=A1; Aloe=A2

The δ^{13}C values for A1 and A2 honey samples were -13.66‰ and -13.11‰, respectively and fell in the set range of -11‰ to -13.5‰ of CAM plants (Chen, et al., 2019). However, A1 honey -13.66‰ fell just outside the range. This can be attributed to fact that CAM plants utilise both the C$_3$ and C$_4$ photosynthetic cycles and the δ^{13}C value is mostly affected by water availability (Vetrova, Kalashnikova, Melkov, & Simonova, 2017). If bees collect more nectar or sap from C_4 plants such as sugar cane exudates or from CAM plants, over and above nectar from C_3 plants, the resulting honey they produce may have $\delta^{13}C$ ratios higher than -23.5‰, but care should be taken in interpretation, as such honey may not necessarily be an adulterated honey (Eshete, 2019a). This is particularly relevant for southern African honeys of multifloral or aloe origin and will require further research.

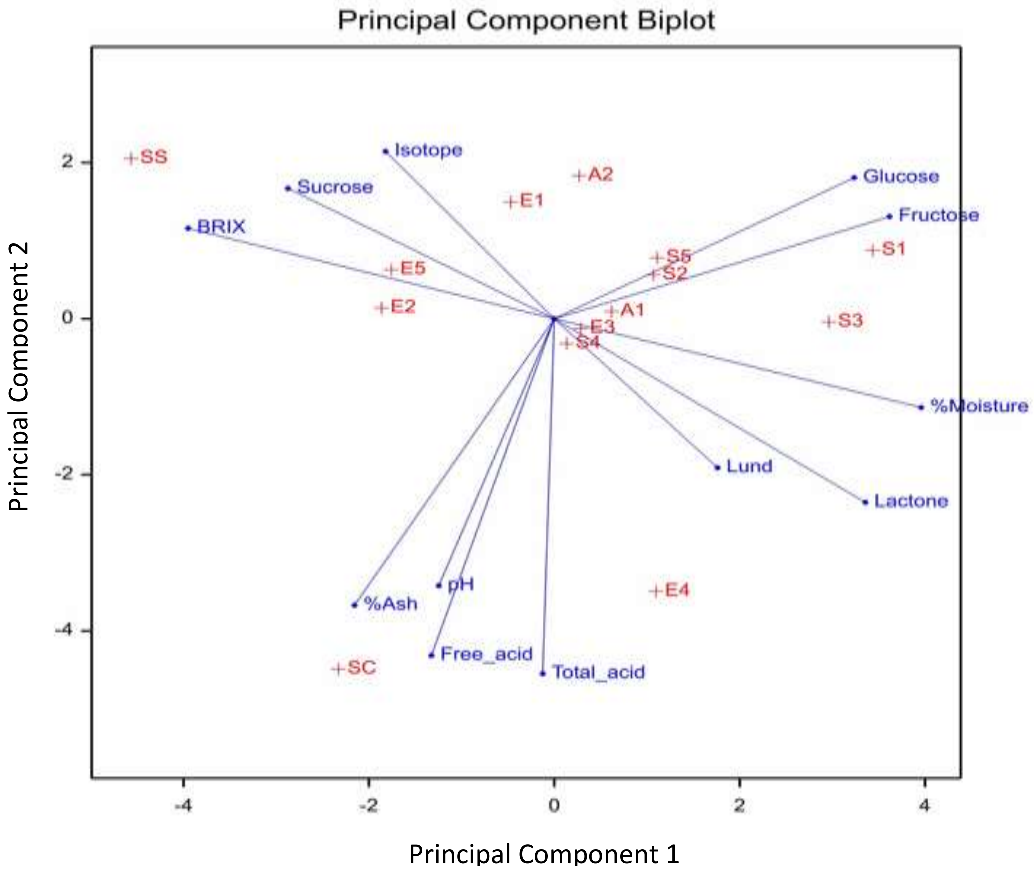

Figure 4.2: PCA analyses showing plot of mean scores for different forage types and sugarcane and sugar syrup Eucalyptus=E1; Eucalyptus=E2; Eucalyptus=E3; Eucalyptus=E4; Eucalyptus=E5; Sunflower=S1; Sunflower=S2; Sunflower=S3; Sunflower=S4; Sunflower=S5; Sugar Cane=SC; Sugar Syrup=SS; Aloe=A1; Aloe=A2

South Africa's rich diversity of plant species provides enough bee forage to produce various types of honey with distinct characteristics (e.g., monofloral and polyflora) (Guelpa, Marini, du Plessis, Slabbert, & Manley, 2017). Since mislabelling of origin is a form of adulteration, it is important to classify and identify the origin of honey in relation to its physico-chemical properties (Fakhlaei, et al., 2020). Consequently, a principal component analysis (PCA) was applied to the isotope evaluation data to identify if any similarities between forage types existed. Figure 4.2.

The first PCA analysis (Figure 4.2) included the two controls, sugar syrup and sugar cane, together with the honey samples. A PCA analysis was done on 12 honey flower (E1, E2, E2, E4, E5, S1, S2, S3, S4, S5, A1 and A2) and two sugar types (SS and SC) to examine the interrelationships of a set of variables, as well as to determine if there is a clear grouping of types and which of the variables included in the analysis distinguished between such groups. The variables included in the analyses were: pH, free acid (meq/kg), lactone (meq/kg), total acid (meq/kg), ash (%), Lund, °BRIX, moisture (%), isotope, sucrose (%), fructose (%), glucose (%), F/G ratio and the L/FA ratio values for each of the 14 types. It excluded RI. The two-dimensional graphical representation (Figure 4.1) accounted for 62.6% and the first three components for 80.0% of the total variation in the data.

The first principal component (PC1 on the X-axis) mainly contrasted SC and SS with the other types and on PC2 (Y-axis) with each other. Sugar Syrup is mostly associated with very high isotope (-10.89) and sucrose (15%) values and a high °Brix (83.56), as well as a low moisture content (14.6%), lactone (1.75 meq/kg) and Lund values (0.0 cm^3) and to a lesser extent, with a low ratio value. Sugar Cane is mostly associated with high free acid (31.87 meq/kg), total acid (37.87 meq/kg) and ash values (0.692%) and is most similar to E4 (raw honey from Sabie with the lowest isotope value of -24.87). S4 and E3 are the two most similar types with total acid value (22.75 meq/kg) and moisture values of 17.5% and 17.6%, respectively. The lactone values for S4 and E3 are 5.25 meq/kg and 5.50 meq/kg, respectively. The scores found for each of the PCs were then correlated with the original variables to identify those that are the most important in discriminating between types. The variables that distinguished most clearly between types for PC1 on the X-axis were L/FA ratio (r=-0.89), fructose (%) (r=-0.83), moisture (%) (r=-0.81), °BRIX (r=0.81), lactone (meq/kg) (r=-0.72) and to a lesser extent glucose (%) (r=-0.68) and sucrose (%) (r=0.66). The variables that distinguished most clearly between types for PC2 on the Y-axis were total acid (meq/kg) (r=0.88), free acid (meq/kg) (r=0.81) and to a lesser extent ash (%) (r=0.67) and pH (r=0.66).

PCA analysis presented in Figure 4.3 was done on the 12 honey flower samples (E1, E2, E2, E4, E5, S1, S2, S3, S4, S5, A1 and A2), excluding SS and SC, to examine the interrelationships of a set of variables, as well as to determine if there is a clear grouping of types and which of the variables included in the analysis distinguished most clearly between such groups. Physico-chemical analysis to evaluate the quality of honey is divided into the following groups namely 1) maturity, which includes analysis of reducing sugars, moisture, apparent sugars; 2) degree of deterioration, which includes analysis of free acidity, diastase activity, HMF; 3) purity, which entails analysis of solids insoluble in water, minerals, ash and pollen, and 4) adulterants assessment, which includes the Legol-, Lund- and Fiehe tests (Salazar, de Freitas, da Luz, Bersch, & dos Santos Salazar, 2017).

The variables included in the analysis were the same as for the PCA analysis (Figure 4.2). The first principal component (PC1 on the X-axis) mainly contrasted E4 from the other types of samples. E4 is mostly associated with ash value (0.713%), free acid (20.87 meq/kg) and total acid (29.87 meq/kg). E3 and S4 are the most similar with a pH-value of 3.64 for E3 and an ash-value of 0.310% for S4. Figure 4.2 shows that S5 and S2 are the more similar in the lower right quadrant with the PC1-axis being positive in both cases and negative on the PC2-axis. S5 is associated with an isotope value of -23.31‰ and S2 has a L/FA ratio of 0.652 meq/kg.

The two-dimensional graphical representation (Figure 4.3) accounted for 59.9% and the first three components accounted for 74.0% of the total variation in the data. The scores found for each of the PCs were then correlated with the original variables to find those that are the most important in discriminating the physico-chemical quality parameters. The origin of sample E3 was from Mpumalanga, while S4 originated from the North West. S5 and S2 originated from Limpopo and Mpumalanga, respectively. The variables that distinguished the most between honey types were percentage (%) moisture (r=0.89), °Brix (r=-0.89), glucose (r=0.88), lactone (r=0.86), percentage (%) ash (r=0.86), free acid (r=0.84), pH (r=0.72) and to a lesser extent total acid (sum of free acid and lactone (r=0.67), L/FA ratio (r=0.67), sucrose (r=-0.63) and fructose (r=0.63).

The moisture content of honey plays a role in maturity, purity, fermentation and honey preservation. In addition, honey has a hygroscopicity characteristic which is due to the high sugar content, mainly fructose, which absorbs moisture from the environment. Both the moisture and °Brix value is significant at p=0.05. °Brix has a strong negative association with the moisture content. The strong negative correlation between moisture and °Brix is caused by the fact that if the moisture content decreases, the °Brix values increase (Machado De-Melo, Almeida-Muradian, Sancho, & Pascual-Maté, 2018). The relationship between the low moisture content (14.6-21.7), high refractive index (1.482-1.500) and °Brix values (76.4-83.6) is evident.

If honeybees use wide variety of botanical sources to produce honey, this leads to variation in the sugar content. Sugar profiles can be used to identify the botanical origin of monofloral honeys, as well as play a role in their maturity. The sucrose value has a moderate, but significant negative association at p=0.05. High sucrose values are related to the botanical origin, maturity, and nectar flux of honey, or indicate artificial feeding (Al-Farsi, et al., 2018; Machado De-Melo, Bicudo de Almeida- Muradian, Sancho, & Pascual-Maté, 2018). A low sucrose value indicated that the honey was matured when harvested. Sucrose is broken down to glucose and fructose. Glucose is partially oxidised by glucose oxidase to gluconic acid and hydrogen peroxide, decreasing the glucose content. This enzyme stays active after processing and results in a decrease in pH and an increase in acidity during storage (Osuagwu, Oyerinde, Onipede, & Ombugadu, 2020). The fact that glucose is less soluble than fructose contributes to the increased tempo of granulation. In certain types of honey such as rape and dandelion, the glucose value is higher and this has an impact on the honey flavour, as fructose is sweeter than glucose.

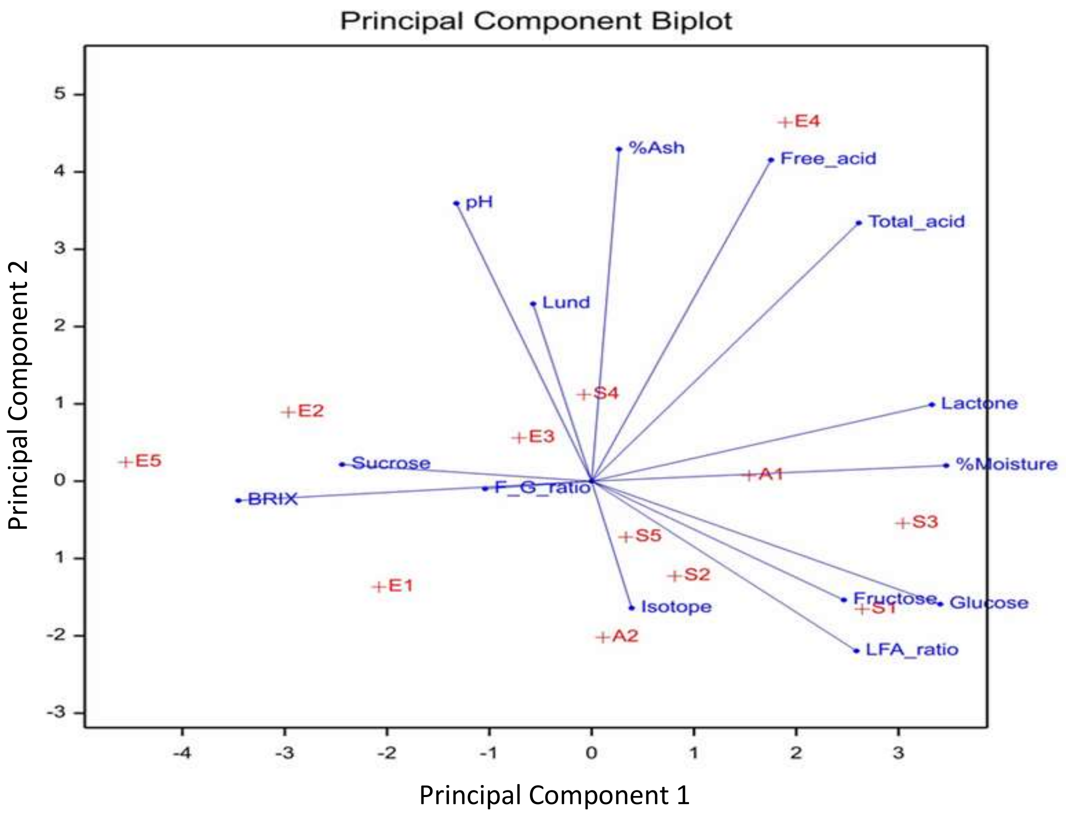

Figure 4.3: PCA analyses showing plot of mean scores for different forage types of Eucalyptus=E1; Eucalyptus=E2; Eucalyptus=E3; Eucalyptus=E4; Eucalyptus=E5; Sunflower=S1; Sunflower=S2; Sunflower=S3; Sunflower=S4; Sunflower=S5; Sugar Cane=SC; Sugar Syrup=SS; Aloe=A1; Aloe=A2

The F/G content shows a weak negative significance at p=0.05 (Al-Farsi, et al., 2018; Machado De-Melo, Bicudo de Almeida- Muradian, Sancho, & Pascual-Maté, 2018). This ratio can play a role in the classification of monofloral honeys or for detecting adulteration with glucose and high-fructose syrups (Aljohar, et al., 2018). Furthermore, the F/G ratio is characteristic of certain honeys, for example, rape honey (*Brassica napus*) has a higher glucose value than other honeys. In a study done by Szczęsna and coworkers, 2011 found higher glucose values in rape honey samples, than for fructose. The F/G ratio is also indicative of the crystallisation rate of honey. The higher the F/G ratio (>1.3), the higher the tendency of the honey to remain in a liquid form, while a low F/G ratio (<1.0) increases the crystallisation rate. These researchers found a glucose range of 32.3-40.7 g/100 g compared to the fructose range of 31.9-40.3 g/100 g, hence the F/G ratio varied from 0.88-1.13 (Szczęsna, Rybak-Chmielewsk, & Teper, 2011). These low F/G ratios (<1.0) should be taken into consideration in legislation, where applicable. Moreover, this low F/G ratio (<1.0) is not compliant with the APS Act (DALRRD, 2000).

The acidity of honey is not related to geographical origin, however it is due to the presence of organic acids, particularly gluconic acid. The acidity of honey plays a role in deterioration. With lactone responsible for the neutralisation of honey from acidic to alkaline. Free acid increases with time and is due to spoilage, while lactones account for the acidity. Though lactones are not constant, their hydrolysis also increases free acid content (Osuagwu, Oyerinde, Onipede, & Ombugadu, 2020). Low values of free acid indicate ripeness of honey when harvested. Free acid content can be attributed to the sugar concentration of the nectar, as well as, seasonal variation, harvest time, geographical location, extraction and storage. Furthermore, it is an indication that the honey sample has possibly been blended with either a monofloral or polyfloral honey (Eshete, 2019a; Warui, et al., 2019). In addition, the pH of honey is not directly related to the acidity due to the buffer capacity of salts and certain mineral compounds (Machado De-Melo, Almeida-Muradian, Sancho, & Pascual-Maté, 2018). The variation in the pH-values of the different honeys depends on the amount of organic acids the honeys contain, their botanical origin (blossom and honeydew), ionized minerals that are present, the extraction and storage (Osuagwu, Oyerinde, Onipede, & Ombugadu, 2020).

The differences in physico-chemical parameters in the honeys can be attributed to their botanical origins and the geographical regions from which they originated. This study included five provinces of South Africa, namely North West, Natal, Gauteng, Mpumalanga and Limpopo, one imported sample produced in Australia, as well as a SC and a SS. Other factors contributing to the variation in the isotopic ranges are the source geographical location, the harvest time, and whether the honey was raw or had been commercially processed (Se, Wahab, Yaacob, & Ghoshal, 2019). All samples were raw except the sugar cane, one *Eucalyptus* sample and the imported honey that has been filtered. During the filtration process impurities are removed as well as pollen, which influences the determination of botanical origin. However, it is currently not possible to determine if the pollen has been added, or not (Fakhlaei, et al., 2020).

In determining the botanical and geographical origin of the 14 honey samples melissopalynological and stable isotope analyses were performed. A screening test was performed to determine the results of melissopalynological analysis as presented in Table 4.4. The pollen analyses of the 14 honey samples yielded 52, mostly dicotyledonous, pollen taxa/types. In addition, 13 pollen types were identified only to the family level. Growth forms of the parent plants included trees, shrubs, herbs, climbers, grasses, and sedges. In total, dominant taxa were *Eucalyptus* (eucalypts, E1, E2, E3, E4, E5, SC, SS), *Helianthus* (sunflower, S1, S2, S3, S4, S5) and *Aloe* (A1, A2). Pollen contents of honeys and syrups were classified in categories: Dominant pollen (>45%), secondary pollen (16-44%), minor pollen (3-15%), rare pollen (<3%), and very rare pollen (<1%) (Johannsmeier, 2016). According to pollen analysis, E1, E2, E3, E5, SC, SS are unifloral/monofloral *Eucalyptus* honeys. E4 is dominated by *Eucalyptus,* but regarded as multiflora honey. S1, S2, S3, S5 are unifloral *Helianthus* honeys. S4 is dominated by *Helianthus*, but regarded as multifloral honey. Most of the honeys and syrups, with exception of the *Aloe* honeys (A1, A2) reflect a vegetation dominated by crops (*Helianthus*, *Eucalyptus*, Brassicaceae, *Citrus, Trifolium,* etc.) and weeds (e.g., Plantago).

The results indicated that bees forage on different plant species from both natural and agricultural ecosystems. Most of the honey samples were correctly identified as indicated on their labels, however the S4, SC and SS dominant taxa were *Eucalyptus* indicating that the environment where the bees foraged were dominated by *Eucalyptus* plants. E3 and SC was identified as botanically not correctly named. E3's pollen composition indicated that it is a multifloral honey with a strong *Helianthus* contribution, not an *Eucalyptus* honey*.* The SS sample was not botanically linked to any botanical honey type; hence it was correctly labelled by the beekeeper as sugar syrup product. The pollen analysis indicated that the SS sample contained mostly *Eucalyptus* honey. This is probably due to a shortage of *Eucalyptus* nectar flow, forcing the beekeeper to provide additional feeding in the form of a sugar solution. The high sucrose (15%) content confirmed the additional feeding. This is a clear example of indirect adulteration of a honey product and was clearly labelled as such. Geographical origin was not determined, although some pollen indicators reflect the floral species where the nectar has been collected (Warui, et al., 2019).

Stable carbon isotope analysis may detect potential adulteration with C_4 plant sugars, and it can be used to determine the botanical and geographical origins of honey and sugary solutions (Eshete, 2019a). The determination of the carbon isotope ratio ($\delta^{13}C$ /^{12}C) and the degree of C_4 sugar adulteration (%) have been accepted as the means to detect C_4 adulteration. The addition of C_3 sugars cannot be detected via carbon isotope analysis alone and additional tests are required to determine potential adulteration (Eshete, 2019a). The types of adulterants used may also depend on factors such as geographical location, cost effectiveness and availability of the favoured adulterants (Fakhlaei, et al., 2020). European countries predominantly use fructose inulin syrups, whereas in China, preferred adulterants such as rice and maltose syrups are used. These are favoured due to the difficulty to isotopically detect adulterants of C_3 origin (Eshete, 2019a; Se, Wahab, Yaacob, & Ghoshal, 2019). The carbon isotope analysis alone was not sufficient to determine potential adulteration. The South African honeys with $\delta^{13}C$ values between -23.5‰ and -21.5‰ fall within a grey area, as the carbon isotope ratios obtained for these samples suggest that these products may be adulterated to a degree, whilst those with a $\delta^{13}C$ value of ≥ -21.5‰ may be deemed to be adulterated (Eshete, 2019a).

The following honey isotope compositions all fall within the grey area: SC (-22.41‰), E5 (-21.76‰), E2 (-23.22‰), E3 (-23.27‰), S4 (-22.18‰), S3 (-23.43‰), S1 (-23.47‰) and S5 (-23.10‰). In addition, the SC sucrose (%) value was above 5%, while in samples E5 and E2 the reducing sugars (%) were below the set limit of 65%. The S1 and S3 samples did not comply with the defined moisture limit. In contrast, the E3, S4 and S5 samples complied with all physico-chemical parameters. Adulteration with C_3 sugars produced from beet, rice or wheat cannot be isotopically detected using only carbon isotope analyses of bulk honey (Fakhlaei, et al., 2020). Hence, development is the methods using the carbon isotope ratios of bulk and extracted protein.

Out of the 14 honey samples, six honey samples complied with the physico-chemical parameters (sucrose, reducing sugars, F/G ratio, pH, total acidity, moisture, Lund's precipitate and ash), as defined in the APS Act (DALRRD, 2000), indicating that these products may be considered to be true honey. If a honey complies with legislation in terms of its composition and labelling requirements it is considered a true honey (Fakhlaei, et al., 2020). The SC did not comply because it exhibited low reducing sugars (53.4%) and carbon isotope values (-22.41‰). The reducing sugars values of both E2 and E5 were below 65%, while the E2 and E5 carbon isotope values were -23.22‰ and -21.76‰, respectively. The carbohydrate composition of honey is one of the key factors in establishing its botanical origin and, indirectly, contributes to its correct classification and identification of [trace] adulteration (Fakhlaei, et al., 2020). While melissopalynology is still the method of choice for confirming botanical origin, it is not able to detect the addition of harvested pollen to the honey to disguise fake honey for the purpose of mislabelling the floral origin of the honey (Fakhlaei, et al., 2020).

The current limitations to this study include the following:

1. The small sample number (n=14) which was due to a shortage of honey and seasonal variations of the type of honey available.
2. Available funding only allowed for certain analyses to be performed.
3. Sensorial and several additional physico-chemical parameters, such as HMF and enzymes analyses were not carried out. The combination of pollen analysis, sensorial and other physico-chemical parameters that complement each other are used for botanical authentication, as well as classification of honey (Fakhlaei, et al., 2020).

Table 4.4: Pollen composition of honey from different regions in South Africa and Australia (E5), respectively

Honey identification number	Taxa spectra	Conclusions
E1	Well preserved pollen of *Eucalyptus* (neophyte)-**dominant**, *Lychnophora*-type (daisy family), Liliaceae (lily family), Casuarina (Australian neophyte), sunflower (*Helianthus*), Gentianaceae, Poaceae (grass family), single fungal spore	Eucalypt honey
E2	Well preserved pollen of Eucalyptus (neophyte)-dominant, sunflower (Helianthus), Rosaceae (rose family), Tubuliflorae (daisy family), Cladosporium (fungal spore)	Eucalypt honey
E3	Minor pollen, but diverse: sunflower (Helianthus), slightly dominant, Eucalyptus, Poaceae (grass), Tubuliflorae (daisy family), Dodonaea (indigenous woodland tree), Plantago (plantain, weed), Stoebe/Elytropappus (daisy family), Scabiosa (herb)	Multifloral honey with strong *Helianthus* contribution
E4	Well preserved pollen of Eucalyptus (neophyte), no other taxa detected	Pure Eucalypt honey
E5	Well preserved pollen of Eucalyptus (neophyte)-dominant, Cardamine (weed, cabbage family) also strong!, strong: Echium (introduced weed), weak: Citrus (lemon/orange)	Eucalypt-*Cardamine* honey
S1	Well preserved pollen of Helianthus (sunflower)-dominant, much lower: Poaceae (grass), Senegalia/Varchellia (acacia trees), Gentianaceae, Atriplex (goosefoot family, weed), Plantago (plantain, weed), Rosaceae (rose family), Cardamine (probably introduced weed, member of the cabbage family/Brassicaceae), Trifolium (clover), Cyperaceae (sedge), Ambrosia (neophytic herb, ragweed)	Sunflower honey
S2	Well preserved pollen of Helianthus (sunflower)-dominant, much lower; Ambrosia (ragweed), Atriplex (weed, goosefoot family), Commelinaceae, Gentianaceae, Tubuliflorae (daisy family), Citrus (cultivated neophyte, lemon/orange)	Sunflower honey
S3	Well preserved pollen of Helianthus (sunflower)-dominant, Malvaceae (e.g., Hibiscus), Tubuliflorae (daisy family), Quercus (oak), Zea mays (maize), Ambrosia (ragweed), Poaceae (grass), Vitis (grape, cultivar), Ligustrum (introduced shrub)	Sunflower honey
S4	Well preserved pollen of Helianthus (sunflower) & Eucalyptus (neophyte)-dominant, but sunflower a bit stronger, Strong: Tubuliflorae (daisy family), Poaceae (grass), Persicaria (aquatic), Vitis (grape), Casuarina (Australian tree), Senegalia/Varchellia (acacia tree), Crassula, Plantago (plantain, weed), Fabaceae (bean family), Pentzia (shrublet-daisy family), Olea (olive tree), Torula (fungal spore)	Sunflower-Eucalypt honey (but with contribution by daisy family)
S5	Well preserved but not very abundant pollen of: Helianthus (sunflower)-dominant, moderately strong: Poaceae (grass), Tubuliflorae (daisy family), weaker: Olea (olive tree), Protea, Mentha (mint family), Ambrosia (ragweed), Eucalyptus, Combretaceae (indigenous woodland trees), Alternaria (fungal spore)	Sunflower honey
SC	Well preserved and very abundant pollen of Eucalyptus (neophyte)-strongly dominant, few of Helianthus (sunflower), Tubuliflorae (daisy family), Ambrosia (ragweed, neophytic weed), Carryophyllaceae (carnation family), Senegalia/Varchellia (acacia trees), Poaceae (grass)	Eucalypt honey
SS	Well preserved pollen of Eucalyptus (neophyte)-dominant, moderately strong: Tubuliflorae (daisy family), Ericaceae (heath family), weaker: Senegalia/Varchellia (acacia trees), Citrus (lemon/orange), Lilaiceae (lilies), Searsia (woodland tree), Helianthus (sunflower), Brassicaceae (cabbage family), Tubuliflorae (daisy family), Carryophyllaceae (carnation family), Ulmus (elm tree, neophyte), Fabaceae (bean family), Plantago (plantain, weed), Selago, Ruschia, Lychnophora (daisy family), Crassula, Brassicaceae (cabbage family, Biscutella (cabbage family, introduced weed), Poaceae (grass)	Eucalypt honey with strong contribution of daisies and heath, very diverse
A1	Well preserved pollen of Aloe (lily family)-strongly dominant, few of Eucalyptus, Searsia (indigenous woodland tree)	*Aloe* honey
A2	Well preserved pollen of Aloe (lily family)-strongly dominant, few pollen of Helianthus (sunflower), Crassula, Tubuliflorae (daisy family), Olea (olive tree), Poaceae (grass), Senegalia/Varchellia (acacia trees)	*Aloe* honey

Eucalyptus=E1; *Eucalyptus*=E2; *Eucalyptus*=E3; *Eucalyptus*=E4; *Eucalyptus*=E5; Sunflower=S1; Sunflower=S2; Sunflower=S3; Sunflower=S4; Sunflower=S5; Sugar Cane=SC; Sugar Syrup=SS; Aloe=A1; Aloe=A2

Honey quality is determined by multiple factors including, geographic and botanical origin, climatic conditions, bee species, as well as postharvest production practices. Low quality honey can negatively influence the authentication of a honey, as well as its reputation, which is why pure honey is in high demand and commands an important sector of the market (da Silva, Gaunche, Gonzaga, Costa, & Fett, 2016; Soares, Amaral, Oliveira, & Mafra, 2017). In verifying the botanical and geographical origins a combination of various methods, such as physico-chemical, melissopalynology and carbon isotope testing are necessary. However, little melissopalynology information is available for southern African honey. In South Africa detecting adulteration by means of added sugars with carbon isotope analysis is currently limited because only bulk honey samples are analysed without protein extraction.

The geographic origin of the South African honey samples could not be isotopically confirmed yet. It will be necessary to establish a database based on multiple isotope ratios (carbon, nitrogen, oxygen and hydrogen), pollen analyses and measurements of the aforementioned physico-chemical parameters of southern African honey samples collected from multiple locations and over a number of years. This long-term project would allow the determination of the range of geographic, botanical and seasonal variability reflected in southern African honeys.

4.5 Conclusions and Recommendations

The authenticity of honey remains difficult and is a challenge because of the matrix complexity and the various methods of adulteration. It is, therefore, advantageous to perform as many analyses as possible to verify the authenticity of honey, as no single method exists to confirm authenticity. The quality of honey is mainly determined by its sensory, chemical, physical, and microbiological values/parameters with the required composition criteria. To decide if a honey is unifloral or not, is based on a global interpretation of all the analytical results that are available. It should also be kept in mind, that honey can acquire different characteristics and properties depending on its environment of the geographical location of the hives are located and the surrounding environment.

4.6 Credit Statements

Tersia de Beer: Conceptualisation, Methodology, Software, Formal Analysis, Resources, Investigation, Writing - original draft.

Beulah Pretorius: Supervision, Conceptualisation, Writing - Review and Editing.

Hettie Schönfeldt: Project administration, Supervision, Conceptualisation, Visualisation, Review and Editing, Funding acquisition.

Grant Hall: Stable isotope methodology and analyses. Writing - review & editing.

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4.8 Conflict of interest

The authors declare no conflict of interest.

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CHAPTER 5: LEGISLATION VS REGULATIONS

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5.1 Abstract

Legislation, standards, and food safety systems play a significant role in the management of a food commodity in the food industry. Governments make decisions, publishing policies, regulations, as well as implementing legislation that sets minimum food and marketing compliance standards by the food industry. The honey value chain is currently regulated by a limited number of international standards, and the import and export of this product are currently mainly based on the parameters set out in the Codex Alimentarius. In this chapter, an overview of the regulatory framework governing the honey trade globally is presented with the focus on the South African Agricultural Product Standards Act (APS), 1990 (Act No 119 of 1990). It has been established that in the regulatory framework in South Africa, fundamental problems/challenges prevail. In order to address these challenges, the regulations need to be revised by cross sector collaboration on a more regular basis with a specific focus on what is relevant to South African honey.

Keywords: legislation, regulations

5.2 Introduction

Authenticity of honey is a major issue in the global honey market as it becomes more and more prone to adulteration due to honey's relatively high price and unique properties. For example, the botanical/flower origin of honey is of great importance in food analysis, since authenticity guarantees its quality (Machado, Miguel, Vilas-Boas, & Figueiredo, 2020). Honey is a complex nutritional sweetener with a diverse composition which is affected by various natural factors (e.g., climatic conditions, floral sources which provide nectar and pollen, geographical origin) and human factors (e.g., knowledge about harvesting conditions and technology, handling, processing and storage). Furthermore, bee species and sophisticated adulteration processes also play a role (Chin & Sowndhararajan, 2020).

There are various ways of adulterating honey, that include feeding honeybees sugar syrups during nectar flow or by adding sugars/syrup to the honey. Other forms of adulteration are the mislabelling of honey according to botanical and geographical origin, heating to prevent delay in crystallisation and dyeing of honey with sulphite-ammonia caramel to obtain more favourable colours (Makowicz, Jasicka-Misiak, Teper, & Kafarski, 2018; Bodor, et al., 2020). Hence, quality control and safety protocols of honey have become the centre of attention. Food control is a mandatory regulatory activity enforced by national and local (regional) authorities during production, handling and processing, storage and distribution to ensure that food is safe and fit for human consumption, conforms to quality requirements and is correctly labelled (Makowicz, Jasicka-Misiak, Teper, & Kafarski, 2018). An impairment in the monitoring of quality is the variation in regulations, as countries are issuing national provisions, decisions and guidelines in addition to international legislations as part of the controlling process of honey (Chin & Sowndhararajan, 2020).

Currently, the complexity of global honey trade legislation enhances the difficulty of applying these regulations, making it a necessity for governments to make standards uniform to minimize trade barriers between countries (Adamchuk, et al., 2020). Moreover, these regulations are necessary to ensure fair trade control and products of high quality (Thrasyvoulou, et al., 2018). The Codex Alimentarius Standards for honey, European Directive (EU) and Agricultural Product Standards (APS) Act contain requirements to ensure a sound, wholesome honey product free of adulteration, correctly labelled and presented (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002). These requirements include the specification of honey type, composition criteria, as well as the guidelines on labelling, presentation and classification based on origin (Choi & Nam, 2020). Honey quality criteria specified in the Codex Alimentarius Standard for honey (CODEX STAN 12-1981), is an example of an international standard, while the European Directive 2001/110/EC (EU Directive) is a regional standard. In addition, the South African Products Standards, (APS) Act, 1990 (No. 119 of 1990) is a national standard (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002). However, the regulations differ regarding the physico-chemical, organoleptic and microscopic characteristics as defined according to region, national set standards and the application thereof (Thrasyvoulou, et al., 2018).

In 1981, the Codex Alimentarius Commission adopted the Codex Standard for honey which was subsequently revised in 1987 and 2001. This voluntary application (by government and commercial partners) is the basis for many legislations (Codex Alimentarius, 2001). The European Council adopted the Codex standards and issued the European Directive 2001/110/EC (amended 2014/63/EU), that stipulates the production and trading parameters of honey in the countries that are members of the European Union (EU) (European Commission, 2002; European Commission, 2014). All member states of the EU need to comply with this EU directive. Examples of countries that are not members of the EU where honey standards are in full compliance with the EU directive standards are Bulgaria, Cyprus and England (Thrasyvoulou, et al., 2018). In addition, the Codex standards form the basis of the South African APS Act regulations (DALRRD, 2000; Codex Alimentarius, 2001).

Characterisation of honey origin and description of physical types (e.g., natural, raw, organic and unheated) became a major issue in the entire honey supply chain due to increased demands from consumers based of its pharmacological properties, high cost production and general shortage of honey (Trifković, Andrić, Ristivojević, Guzelmeric, & Yesilada, 2017; Fakhlaei, et al., 2020). Currently, there is a lack of effective methods to monitor/regulate adulterated honey products. Several actions are needed to address and resolve fraud, hence research on the development of reliable methodologies of chemical markers that may contribute to honey characterisation has been increased (Fakhlaei, et al., 2020). The International Honey Commission (IHC), established in 1990, was created with the goal to compile new global honey standards while also encouraging the development of harmonized analytical methods to assess the quality of various honeys (Machado, Miguel, Vilas-Boas, & Figueiredo, 2020). These methods should be characterized by high accuracy and precision, be affordable and produce results as soon as possible (Jakubik, Borawska, & Socha, 2020).

In addressing the challenges attributed to adulteration practices, regular revision of standards, methodologies and regulations is necessary. Therefore, the Codex Standard for honey and EU Directive for honey analysis are continually revised to keep up with fraudulent practices. However, no actual long-term solution to control the production and adulteration of honey has been found. Factors contributing to the lack of solutions include the difficulty in governing quality standards such as variation and the confounding manner in which the standards are compiled (Thrasyvoulou, et al., 2018). A shortcoming of these standards is that, although the honey can meet the minimum quality requirements, it still does not imply that the honey is natural and authentic. Therefore, to authenticate a food product such as honey, it should be indicated that it is real, true and original. Therefore, honey should comply with legislation in terms of its composition and labelling requirements (Choi & Nam, 2020). The availability of synthetic forms of some natural components such as sugar, hydroxy-methyl-furfural (HMF), diastase etc., on the market increase the possibility of adulteration (Dugalić-Vrndić, Kečkeš, & Mladenović, 2011).

This chapter focus on the current legislation and standards governing the monitoring of the quality of honey on the South African market (locally and imported). It is not a comprehensive analysis of public and private sector investments in extensions, research and developments, but rather represents information that demonstrates the need to revise outdated legislation in South Africa. To achieve the main objective of the current study, available literature was reviewed to answer the following research questions:

a) Why is there a need for collaboration between different public entities?

b) Why is there a need for revised legislation?

5.3 South African Context

5.3.1 Food laws

Food laws are divided into two parts: (1) a basic food act and (2) regulations. The Act itself sets out broad principles, while regulations contain detailed provisions governing the different categories of products that fall under the jurisdiction of each set of regulations. In South Africa, the agricultural sector has legislations in place to safeguard the local industry. The South African food and agricultural import regulations and standards are encompassed within at least fourteen Acts that are administered and enforced by three ministries, namely: the Department of Health (DoH), the Department of Agriculture, Land Reform and Rural Development (DALRRD), and the Department of Trade, Industry and Competition (DTIC) and their associated agencies. These appointed independent agencies perform specific regulatory functions. (See Figure 5.1) (Mukumba, 2011).

This multi-agency system and its various divisions inside these ministries are responsible for regulations relating to food labelling and composition. These three (3) departments examine and approve food products before it is placed on the market. Food products that do not comply to the set standards are removed and destroyed in addition the offenders can be prosecuted. The DoH is responsible for health and food safety, DALRRD Agricultural Production, Health and Food safety branch is responsible for food quality and safety for certain agricultural and animal products, and the DTIC is responsible for the compilation of compulsory specifications and standards for various food categories. The responsibilities and functions of each of the regulatory authorities are discussed in more detail in the subsections below. (5.3.1.1, 5.3.1.2, 5.3.1.3).

5.3.1.1 Department of Health (DoH)

The National DoH and the directorates of Food Control and Health Promotion and Nutrition is responsible for food hygiene management, food safety and quality standards development, food labelling, nutrition and fortifications requirements for foodstuffs (agricultural & non-agricultural products as they are released in the market from the jurisdiction of DALRRD or imported into South Africa) available on the market (i.e., after production) (Sekgala, 2018). The Foodstuffs, Cosmetics and Disinfectants (FCD) Act (Act No 54 of 1972) falls under the DoH, with the objective of ensuring that foods consumed are safe and that consumers are given reliable information to aid them in making the correct food choices (DoH, 1972). Under the FCD Act selling unsound, adulterated and contaminated food is a criminal offence. Legal action can be taken against offenders by removing and/or destroying such food product, however laboratory confirmation is needed to pursue further legal action. In addition, the DoH is also responsible for incorporating the Codex Alimentarius Commission standards, guidance and codes of practices into national food safety policies. These include the Sanitary and Phytosanitary (SPS) Agreement and the Hazard Analysis and Critical Control Point (HACCP) food safety management system (Boatemaa, et al., 2019). The Chief Directorate: Health, Promotion and Nutrition of DoH is responsible for the administration of baby food regulations and infant formula regulations, as well as the development of nutritional programmes (Mukumba, 2011; Sekgala, 2018). The food law in South Africa under the DoH is enforced in municipalities by environmental health, practitioners under the FCD Act, 1972 (Act No 54 of 1972) (Mukumba, 2011).

5.3.1.2 Department of Agriculture, Land Reform and Rural Development (DALRRD)

The DALRRD is the leading regulatory authority responsible for food security and Sanitary and Phytosanitary (SPS) issues related to agricultural products (Sekgala, 2018). This is carried out through the Veterinary Public Health, Plant Health, Food Safety and Assurance and Agricultural Products Inspection Services. Food safety issues fall under the branch of Agricultural Production, Health, and Food Safety. Various acts fall under the branch of Agricultural Production, Health, and Food Safety, namely: the APS Act; Plant Improvement Act, 1976 (Act No 53 of 1976); the Plant Breeders‘ Rights Act, 1976 (Act No 15 of 1976); the Veterinary and Para Veterinary Professions Act, 1982 (Act No 19 of 1982); the Animal Diseases Act, 1984 (Act No 35 of 1984); the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act No 36 of 1984); the Liquor Products Act, 1989 (Act No 60 of 1989); the Genetically Modifies Organisms Act, 1997 (Act No 15 of 1997); the Meat Safety Act, 2000 (Act No 40 of 2000); and the Animal Identification Act, 2000 (Act No 6 of 2002) (DALRRD, 1976a; DALRRD, 1976b; DALRRD, 1982; DALRRD, 1984a; DALRRD, 1984b; DALRRD, 1989). The aim of these regulations is to provide products of consistent quality, which is assessed based on set standards for specific food products.

The DALRRD has a twofold function – it is responsible for agronomy, agricultural practices and fresh produce administration, as well as agricultural product standards relating to labelling (grading & marking), imports and exports (Sekgala, 2018). Products which are regulated under the APS Act, include fresh products of plant origin, and products of certain animals. Department of Land Reform and Rural Development regulations are promulgated at National level, but enforced on the Provincial level, but not by the APS Act, as assignees are used (DAFF, 2018). The Meat Safety Act, 2000 (Act No 40 of 2000) and Liquor Products Act, 1989 (No 60 of 1989) oversee imports and exports of animal products related to meat and liquor products, respectively. The sell and production of mead is authorised and regulated under the Liquor Products Act, 1989 (Act No 60 of 1989) (DALRRD, 1989; DALRRD, 2000). Additionally, it also addresses the safety and quality issues of mead products (Sekgala, 2018). While, the South African Meat Industry Company (SAMIC) regulates the meat industry (Boatemaa, et al., 2019).

5.3.1.3 Department of Trade, Industry and Competition (DTIC)

The DITC created the Food Legislation Advisory Group (FLAG)- to address trade issues as well as it is a representative platform between the three departments DoH, DALRRD and DTIC (Boatemaa, et al., 2019). The DTIC includes the Regulatory Compulsory Specifications (NRCS), National Liquor Authority (NLA), South African Bureau of Standards (SABS) and Companies and Intellectual Property Commission (CIPC - Patents, Design and Innovation Division) are responsible for the monitoring and evaluation of the legislation (Mukumba, 2011).

The DTIC enforces food laws under the National Regulatory Compulsory Specifications (NRCS) agency according to the National Regulator for Compulsory Specifications Act, 2008 (Act No 5 of 2008), the Consumer Protection Act, 2008 (Act No 68 of 2008), as well as the Liquor Act, 2003 (Act No 53 of 2003). In addition, it is also responsible for administering the Trade Metrology Act of 1973 (Act No 77 of 1973) and the Trade Marks Act, 1963 (Act No 62 of 1963), which have been replaced by Trade Marks Act, 1993 (Act No 194 of 1993) both of which are concerned with food labelling. The CIPC is responsible for the various acts for patents, designs, trade and merchandise marks, as well as copyrights (Mukumba, 2011). The tradename under which you want your honey to be marketed, is registered, but not the honey product. The National Regulator for Compulsory Specifications Act, 2008 (Act No 5 of 2008) ensures that certain products (e.g., all canned meats, canned and frozen fish and seafood) comply with a set of technical standards. In addition, the Consumer Protection Act, 2008 (Act No 68 of 2008), makes it illegal for consumers to be misled in any way (DTIC, 2008). Furthermore, the South African Bureau of Standards (SABS), falls under the jurisdiction of the DTIC and provides national standards which companies can use as yardsticks to seek certification. Hence, the SABS is mandated to develop, promote and maintain the South African National Standards (SANS), protecting the quality of commodities, products and services and rending conformity assessment services. For the food and beverage industry, SABS assist with assessment, product testing and certification (Mukumba, 2011; Sekgala, 2018).

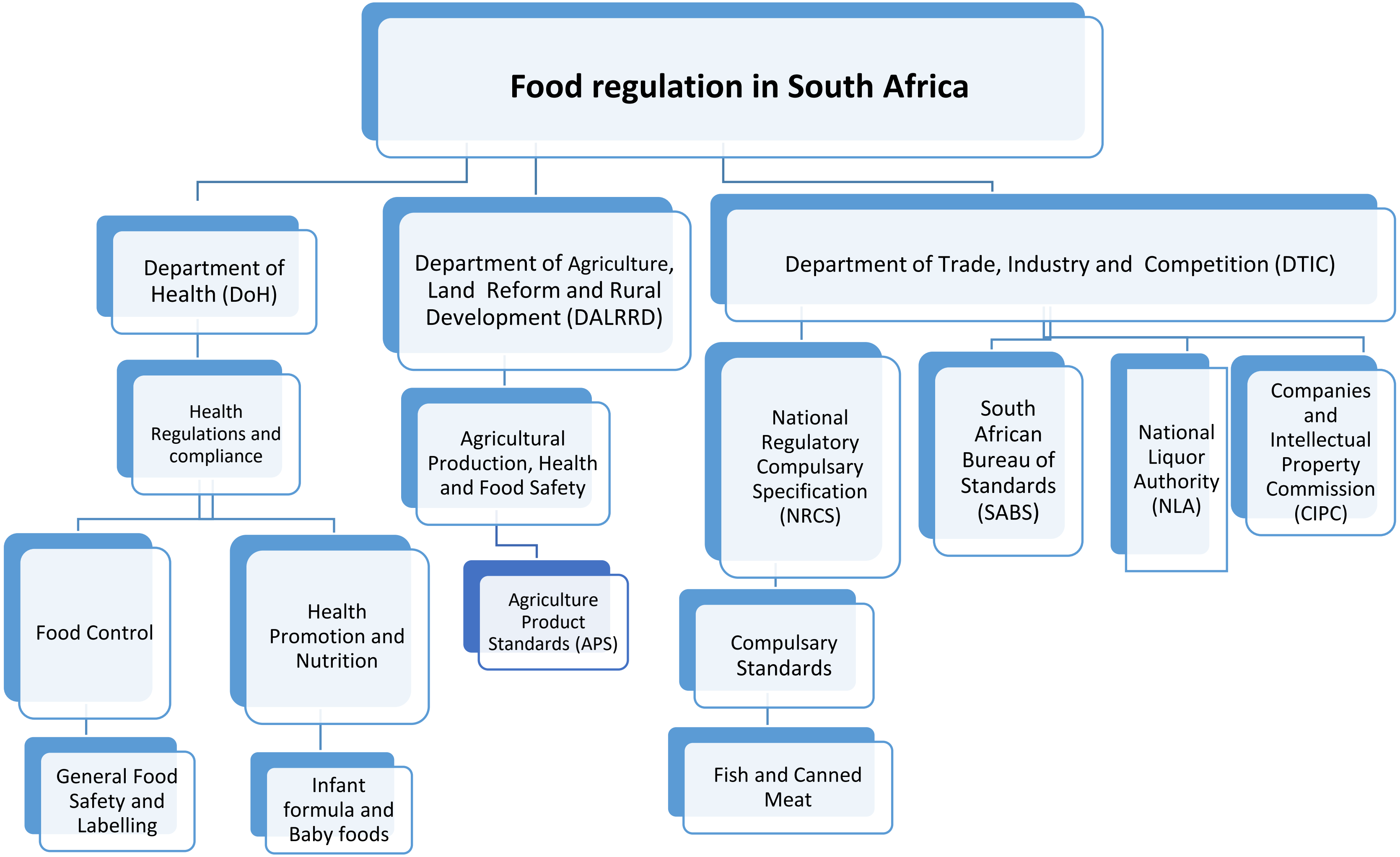


Figure 5.1: The three authorised food regulators in South Africa: The Department of Health (DoH), the Department of Agriculture, Land Reform and Rural Development (DALRRD), and the Department of Trade, Industry and Competition (DTIC), (Mukumba, 2011)

All three regulatory bodies, DoH, DALRRD and DTIC contribute to the grading and labelling requirements of the honey industry in South Africa. The Regulations relating to the Advertising and Labelling of Food stuff (R146/2010) which falls under the Foodstuffs, cosmetics and Disinfectants (FCD) Act (Act No 54 of 1972) and the Consumer Protection Act (No 68 of 2008). Which mandate accurate food labelling and protect consumers from exploitation (DoH, 1972; DTIC, 2008; Boatemaa, et al., 2019).

The following legislation is important in South Africa for the labelling and marketing of honey and mixtures of bee products and any other foodstuff containing honey. Both the Agricultural Pests Act, 1983 (Act No 36 of 1983) and the Foodstuffs, Cosmetics and Disinfectants (FCD) Act (Act No 54 of 1972) acts make provision for the control over safety, labelling and marketing of honey as well as the importation of honey and honey products (DoH, 1972; DALRRD, 1983) The APS Act, *Regulations relating to the grading, packing and marking of honey and mixtures of bee products intended for sale in the Republic of South Africa Regulation. No R. 835 dated 25 August 2000*. The tests to determine the quality, composition, and ripeness of honey are set out in the APS Act and in addition prescribe the requirements for the labeling of honey (DALRRD, 2000). Trade Metrology Act of 1973 (Act No 77 of 1973) which prescribed the correct indication of weight labelling and the Trade Marks Act, 1993 (Act No 194 of 1993) which provide for the registration and certification of trade marks (DTIC, 1973; DTIC, 1993; Mukumba, 2011). However, South Africa lacks official labelling regulations for genetic modified organisms, organic honey and halal food products (Tung, 2018; Boatemaa, et al., 2019).

In summary, various challenges exist in these three ministries to enforce this plethora of food regulations due to the fragmentation of control, lack of coordination when implementing regulations and the capacity constraints to ensure compliance. Challenges experience by DoH include the financial constraints of municipalities to enforce food regulations. At the same time, DALRRD is affected by a shortage of staff to ensure the enforcement of some of its regulations. Likewise, DTIC also experiences challenges in enforcing the proposed regulations. One example is the mandatory labelling of genetic modified organisms (GMOs) regulations. These regulations were withdrawn due to widespread non-compliance of the food industry, as well as the disputation and criticism they received (Tung, Organic food certification in South Africa: A private sector mechanism in need of State regulation, 2016). Other challenges include lack of political will and technical capabilities in the coordination of functions, duplication and sometimes confusion caused by conflicting jurisdictions within and between the government departments mandated to ensure food safety. This contributes to the inconsistent implementation and application of regulations affecting the South African local market and imported food market.

Figure 5.2: Front and Back Labelling requirements as described below, (DoH, 1972; DTIC, 1973; DTIC, 2008; DALRRD, 2000)

The following list demonstrate the roll of each of the regulatory authorities:

Name of the Product – DoH and DALRRD

Food Grading - DALRRD

Irradiated/radiused (imported honey) -DoH and DALRRD

Net Content/Mass – DTIC, DoH and DALRRD

Country of Origin – DoH and DALRRD

Business Name and Address - DoH and DALRRD

Use by date / Date of packing – DOH and DALRRD

Batch Identification – DoH and DALRRD

Additional information

Ingredient declarations (Including List, Font, Nutritional Values, Fat content, Bee Products, allergen) – DoH and DALRRD

Storage Instructions – DOH

Instruction for use – DOH

Definitions of words allowed on food labels – hand-made, farm style, pure, Irradiated, Raw-honey, Unprocessed honey etc - DoH and DALRRD

Indication of Sugar Cane honey Ingredient if applicable – DALRRD

Ingredient declaration (Including List, Font, Nutritional Values, Fat Content, Bee Products – DoH and DALRRD

Mandatory allergen and possible allergen contamination declarations – DoH

5.3.2 South African case study

Honey is a sweet viscous substance produced by honeybees from the nectar of flowers or the secretions of living plants or excretions of plant-sucking insects on plants (Chua & Hamzah, 2020). The rich biodiversity in South Africa favours the production of honey, beeswax and other bee products such as pollen, propolis, royal jelly, bee venom, queen bees etc. Currently, the South African honey industry is dominated by commercial beekeepers and a few smallholder beekeepers (Hans, Taruvinga, & Mushunje, 2018).

The South African honey industry is characterized by underproduction - honey production contributes only 0.11% of the world's total production. This underproduction of honey contributes to South Africa becoming a net importer of honey. It is estimated that South Africans consume 5 000 tons of honey per annum, while production only amounts to 2 000 tons per annum. To meet consumer needs, more than 3 000 tons of honey are imported from China annually (Hendricks, 2021). South Africa's imports of honey increased from 2 000 tons in 2018 to 6 000 tons in 2020, with 4 700 tons (60%) primarily imported from China, but also from other countries including Zambia (706 tons), Poland (305 tons) and Romania (257 tons) (Crewe, Masehela, Human, & Pirk, 2021). Nowadays, the South African honey market is overshadowed by the international demand for honey, which is close to the point where demand cannot be met by global production. The situation opens the door for "artificial honey" or honey replacement products in the marketplace.

It is a challenging task to authenticate honey, as such, food scandals associated with adulterated honey increase the concerns relating to fraudulent practices and increased the importance of authenticating food products (Aljohar, et al., 2018). Several cases of adulterated honey exported from China was exposed during the period of 2008 to 2012. The following types of adulteration practices were exposed transhipment/origin masking, mislabelling, dilution and substitution (Ahmad & Khairatun, 2021).

- Chinese honey was transhipped to other countries namely Indonesia, Malaysia, Mongolia, Thailand, India and Vietnam where it was repacked, and relabelled with fake labels and identity certificates before entering the USA and European markets. This is a clear case of masking the geographical origin of the product. In the industry, melissopalynological analysis is used to confirm honey's geographical origin. (Isopescu, Josceanu, Colta, & Spulber, 2017; Ahmad & Khairatun, 2021).
- Honey that originated from China was mislabelled to circumvent import tariffs and anti-dumping duties by declaring it as rice fructose and not honey when it was exported to the USA. In this case the botanical origin was falsified. In the industry, melissopalynological

analysis is used to confirm honey's botanical origin and sugar profiles are used to determine adulteration with sugars (Isopescu, Josceanu, Colta, & Spulber, 2017; Ahmad & Khairatun, 2021).

- In 2016 EU market research indicated that 14,2% of the 893 jars analysed did not comply to the EU honey standards due to the adulteration of honey with sugars. Although the EU is the second largest honey producer in the world, it still needs to import honey to supplement its domestic demand (Ahmad & Khairatun, 2021). Most of the honey imported into the EU comes from China. To protect the consumer and the European honey market, the Honey Directive 2001/110/EC clearly prescribes the composition criteria for honey. Only products consisting of 100% honey can be sold as "honey" (European Commission, 2002). Adulteration of honey is a form of dilution and authenticity is confirmed with a compositional analysis as prescribed by the EU Directive (European Commission, 2002).

An incident of sugar adulteration occurred in South Africa between July and August 2018.- A manufactured product, high in sugars, was sold on the South African market as honey by a well-known local company. This sugary product was sent to Germany for isotope analysis to determine the $^{13}C/^{12}C$ ratio values to confirm authenticity. Adulteration with added sugars was confirmed, contributing to the non-compliance of the sugar profile. The test results confirmed that this honey product was in fact a sugar solution. The company had to recall the product after the fraud was made public (Ahmad & Khairatun, 2021). These fraudulent practices are carried out during production and processing. Food industries can safeguard themselves by taking part in various collaboration and analytical testing programmes to verify their control systems and certifications for effectiveness, efficiency and trustworthiness.

South Africa, as well as many other regions in the world, adapted its quality parameters that are applicable to its honey market requirements. The APS Act is used to monitor the quality of honey on the South African market (DALRRD, 2000). This includes packaging, marking and evaluation of chemical composition and testing for microbiological contaminants. The mandate of the APS Act, on the local market is only quality, since local food safety related issues are governed by the legislation of the Department of Health (Mukumba, 2011). The first official South African honey standards were compiled through the combined efforts of the Honey Standards Committee, Bill Crisp and Prof Dippenaar (Head of the Agricultural Inspection Services) in 1964 (Crewe, Masehela, Human, & Pirk, 2021). In 1973, the regulation for the selling of honey in South Africa was established (R.69 16/03/73) which prohibits the selling of honey, honey mixtures and honey substitutes unless the honey has been graded, packed and marked as prescribed under the Marketing Act, 1968 (Act No 59 of 1968)

(Department of Agricultural Economics and Marketing, 1973). Table 5.2 is a summary of the physico-chemical parameters with their set limits that are used to determine and evaluate the quality of honey.

South Africa's honey industry is well legislated and regulated but has its own challenges. One challenge is monitoring and controlling of bee products e.g., honey, royal jelly, propolis etc., due to the delegation of responsibilities to different authorities. Another is the working relationship between government and industry such as the one between the South African Bee Industry Organization (SABIO) and DALRRD (SABIO, 2020). The management of honey and beekeeping in South Africa is governed by National legislation and municipal by-laws. Various types of legislation affecting the beekeeping industry have been promulgated, reviewed, and redrafted (SABIO, 2020). The following are examples of such legislations:

5.3.2.1 Beekeeping legislation

South African beekeeping is regulated by the following two acts:

Agricultural Pests Act (Act No 36 of 1983):

The Agricultural Pests Act (No 36 of 1983) focuses on restrictions, and permit requirements for the importation of beehive products (e.g., honey and beeswax) and apiary equipment. Section 3 provides the requirements of import permits for honey importers. It also makes provision for the promulgation of Control Measures for disease control and prevention, as well as for penalties for non-compliance to these requirements (SABIO, 2020). The assigned authority is DALRRD (DALRRD, 1983).

Control Measure GN R858 15 November 2013 – Control Measure relating to Honeybees:

These control measures are promulgated in terms of the Agricultural Pests Act (36/1983) as amended GN R1511, 22 November 2019. This control measure has replaced Control Measure R1674 promulgated in December 1998. The latter compelled all beekeepers to register with DALRRD annually and managed their beekeeping activities in a responsible manner to prevent the spread of bee diseases and control *Capensis (A.m. capensis)* in *Scutellata (A.m. scutellate)* colonies. Furthermore, it also stipulated that Bee Removal Services should also register annually with the Department of Agriculture currently DALRRD (SABIO, 2020).

5.3.2.2 Grading, Packing and Marking of honey products

The sale of honey and mixtures of bee products is regulated in terms of the APS Act (Act No 119 of 1990), Regulation No. R.835 dated 25 August 2000. The regulation prescribes that honey and mixtures of bee products shall comply with the key elements which include, among others, grading, packing and marking requirements. Producers, packers and importers of honey and mixtures of bee products are advised to comply with the local market requirements prescribed in the APS Act. Furthermore, the procedure of how to deal with non-compliances, offences and penalties is also addressed (SABIO, 2020). The assigned authority is DALRRD (DALRRD, 2000).

Food Quality and Importation Requirements – Regulation R146-Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No 54 of 1972). In terms of Regulation R146 dated 1 March 2010 under the FCD Act, 1972 (Act No 54 of 1972), *Regulations Relating to the Labelling and Advertising of Foodstuffs,* statutory requirements have been prescribed in respect of labelling and marketing of food, including honey and honey products. These regulations prohibit the use of false, misleading or negative descriptions on the labelling. It also enforces the requirement of the date of durability that should be indicated on the label as “best before”, “use by” or “sell by”. Honey is exempted from the requirement. The Food Quality and Importation Requirements is also referred to in the FCD Act, 1972 (Act No 54 of 1972), that makes provision for the control of the safety and quality aspects of the manufacture, sale and importation of foodstuffs and labelling thereof. The requirements for irradiation of honey are also included in this Act. It prohibits the sale and importation of honey, which does not comply with the FCD Act, 1972 (Act No 54 of 1972). The DoH is the assigned authority for this Act (DoH, 1972).

The Directorate: Plant Health and Quality of the DALRRD is responsible for drawing up specific phytosanitary requirements for certain products, including imported honey which needs to be irradiated to prevent the spread of the pathogen Paenibacillus larvae. For the importation of honey into South Africa, compliance with the Agricultural Pests Act, 1983 (Act No 36 of 1983), section 3(1) is required. This is to apply to DALRRD for a permit for the importation of Controlled Goods subject to conditions laid down by the authority (DALRRD, 1983). A permit signed by DALRRD should be presented to DoH before commencing with processing and approval of the application for irradiation of imported honey. The assigned authorities responsible for overseeing this process are DALRRD and DoH (SABIO, 2020).

5.3.2.3 Production and Extraction

Honey Production and Extraction – Government Notice R638 of 2018: Regulations Governing General Hygiene Requirements for Food Premises and the Transport of Food, was promulgated in terms of the Health Act, 1977 (Act No 63 of 1977), (DoH, 1977). This Act inter alia ensure that hygienic standards are maintained, and no contamination occurs in locations where food is prepared and packed. This includes the extraction facilities where honey is extracted (SABIO, 2020).

5.3.2.4 Biodiversity and Bee Forage

Biodiversity and Bee Forage; Conversation of Agricultural Resources Act (CARA), 1983 (Act No 43 of 1983) (DALRRD, 1983a). This Act falls under DALRRD and was promulgated to ensure the long-term protection and sustainable use of natural agricultural resources (products produced by the environment), as well as the prevention of erosion and weakening of the country's water sources, the protection of natural vegetation and the combatting of weeds and invader plants. In Regulations, 6 species of eucalypts have been categorised as a Category 2 Invader Plant with a potential to become invasive (SABIO, 2020).

The National Environment Management of Biodiversity Act (NEMBA), 2004 (Act No 10 of 2004) falls under the Ministry of Water and Environmental Affairs (DALRRD, 2004). Its purpose, amongst others, is to provide for the management and conservation of the country's biodiversity and the protection of species and ecosystems. In the NEMBA regulations eucalypts are not listed in the regulatory list of alien or invasive species. This creates confusion as the one Act falls under the Ministry of Agriculture, Land Reform and Rural Development and does not support the other Act which falls under the Ministry of Water and Environmental Affairs (SABIO, 2020).

5.3.2.5 Municipality Laws

In some of the larger metropoles, Municipal By-Laws fall under the Municipal Health Departments. The following South African cities have bylaws for keeping of bees in the metropolitan areas: City of Johannesburg By-Law on the keeping of Bees, City of Tshwane (Pretoria) Metropolitan Municipality By-Law on the Keeping of Bees, Free State Provincial Regulations for the Keeping of Bees. For the Municipal Areas of the Cities of Cape Town, Durban and Port Elizabeth (now Gqeberha), it appears that there are no specific Municipal By-Laws on the keeping of bees in the metropolitan areas of these urban areas (SABIO, 2020).

5.4 Materials and Methods

This paper is a result of a descriptive study to review the current legislation and standards governing the honey trade globally by comparing the EU Directive, the revised Codex Alimentarius Standard and the national standard used in South Africa, the APS Act. In addition, the regulatory bodies in South Africa responsible for regulating the honey trade in South Africa were also discussed and compared.

5.5 Results and Discussion

5.5.1 The role of the different government entities in enforcing the regulations

Food manufacturers, retailers and government authorities have to regularly assess their vulnerability to fraud to put measures in place to minimize it (Mukumba, 2011). Compared to other countries, South Africans food safety legislations are considered technically complex, and often confusing (Nigel Sunley, Sunley Consulting, 2018). The complexity of the regulatory environment in South Africa is evident in the different authorities e.g., DoH, DALRRD and DTIC that are tasked with regulating different aspects of labelling, food safety, and facility design and products standards in the food control system. (Figure 5.3).

In South Africa, bees and bee products (honey, bees wax etc.) are considered plant and plant products and are regulated in terms of the Agricultural Pests Act 1983 for importation (DALRRD, 1983). In the case of exportation, the Animal Diseases Act (Act No 35 of 1984) is applicable (DALRRD, 1984b). Honey is classified as an animal product by the EU (Trifković, Andrić, Ristivojević, Guzelmeric, & Yesilada, 2017), whereas quality evaluation falls under the APS Act. If any bee product e.g., honey, has to be imported, an importer needs a permit based on an evaluation, the quality of which falls under the APS Act (DALRRD, 2000).

Permits are issued to indicate that the products meet specified phytosanitary import requirements and are in conformity with the certifying statement of the appropriate model certificate set by the importing country. In South Africa, the Animal Diseases Act, 1984 (Act No 35 of 1984), provides the legal support for the control of animal diseases to promote animal health and matters concerned with it. In the Animal Diseases Act, 1984 (Act No 35 of 1984), an animal is classified as any mammal, bird, fish, reptile or amphibian which is a member of the phylum vertebrates, including the carcass of such an animal. Whereby, an animal product means any part or portion of, or product derived from, any animal, including any such part, portion or products in any processed form. Bees are invertebrates and are, therefore, not classified as animals. If a beekeeper does, however, want to export honey, an export certificate must be obtained from the country of import and the RSA Competent Authority should clarify that all conditions are met before export may take place. This function is only performed by the Veterinary Services of the Provincial Departments of Agriculture. In most countries of the world, bees and bee issues will fall under Veterinary Services (DALRRD, 1984b). The DoH is responsible for all the tests relating to food safety that play a role in the health of consumers (DoH, 1972).

Existing legislations impacting on the South African beekeeping industry cover, amongst other things, marketing, importation, quality standards of honey and food safety APS Act; Health Act, 1977 (Act No 63 of 1977) and the FCD Act, 1973 (Act No 54 of 1972), disease control and permission to place hives in certain areas (DoH, 1972; DoH, 1977; DALRRD, 1983; DALRRD, 2000). Indirectly, legislations regarding the environment, which has an impact on the beekeepers, are included in the CARA Act, 1983 (Act No 43 of 1983) and the NEMBA, 2004 (No 10 of 2004), as well as the Stock Remedies Act, 1936 (DALRRD, 1983a; DALRRD, 2004). The registration of beekeepers is dealt with in Notice R1674 of the Agricultural Pests Act (DALRRD, 1983).

South Africa’s sanitary management capacity is supported by an extensive regulatory framework with principal Acts in the disciplines of plant- and animal health, as well as food safety, and these are supported by subordinate regulations, strategies and relevant norms and standards (DoH, DTI, & DAFF, 2013). Table 5.1 is a representation of the different Departments' sanitary and phytosanitary legislations that play a role in the South African Food Control System, including the quality of honey. The regulation of food laws is for the most part overseen by the three government departments: DALRRD, DoH and DTIC. Each department functions at national, provincial and local levels, each with its own legislative powers, and is responsible for the implementation and enforcement of many policies, legislation, and regulations. However, this plethora of laws and regulations can increase the risk of overlapping jurisdiction and unnecessary complexity (DoH, DTI, & DAFF, 2013).

5.5.2 Legislations

5.5.2.1 Honey legislations (APS Act, 1990 (Act No 119 of 1990)

The nature of legislation in the DALRRD administrated APS Act (Figure 5.1) is an example of the entanglement of legislation (DALRRD, 2000). Various food products fall under this act e.g., dairy, honey, juices, etc. These regulations cover both compositional and labelling requirements and supersede the DoH's requirements. The advantage of the DALRRD regulations includes the following: good enforcement due to the use of a dedicated inspection function that falls under DALRRD rather than municipalities; DALRRD has a tradition of good and extensive consultation regarding new regulations if the complicated, technical rationale behind compositional requirements is provided. In contrast the disadvantages are technically complex regulations which are often bureaucratic, ambiguous and in many instances out of line with global standards, as well as historically protectionist (to protect domestic industries against foreign competition). Historical baggage has been partially carried over to current systems e.g., actively discourage imports; increase cost of inspections to industry and outsource them to small, micro and medium enterprises (Nigel Sunley, Sunley Consulting, 2018).

In South Africa the legal compositional and labelling aspects that honey producers have to comply with are specified in the APS Act, *Regulations relating to the grading, packing and marking of honey and mixtures of bee products intended for sale in the Republic of South Africa Regulation. No R. 835 dated 25 August 2000* (DALRRD, 2000). Because this Act was last revised in 2000, it is important to evaluate its current validity. From 1973-1979, no specific regulations existed for the regulation of honey production. Specific regulations for compositional criteria, colour and grades were proclaimed in the Government Notice No. R. 2336 – 19 October 1979 (Department of Agricultural Economics and Marketing, 1979). In 2000, both regulations were combined as one regulation, namely R. 835 – 25 August 2000 in terms of the APS Act (DALRRD, 2000).

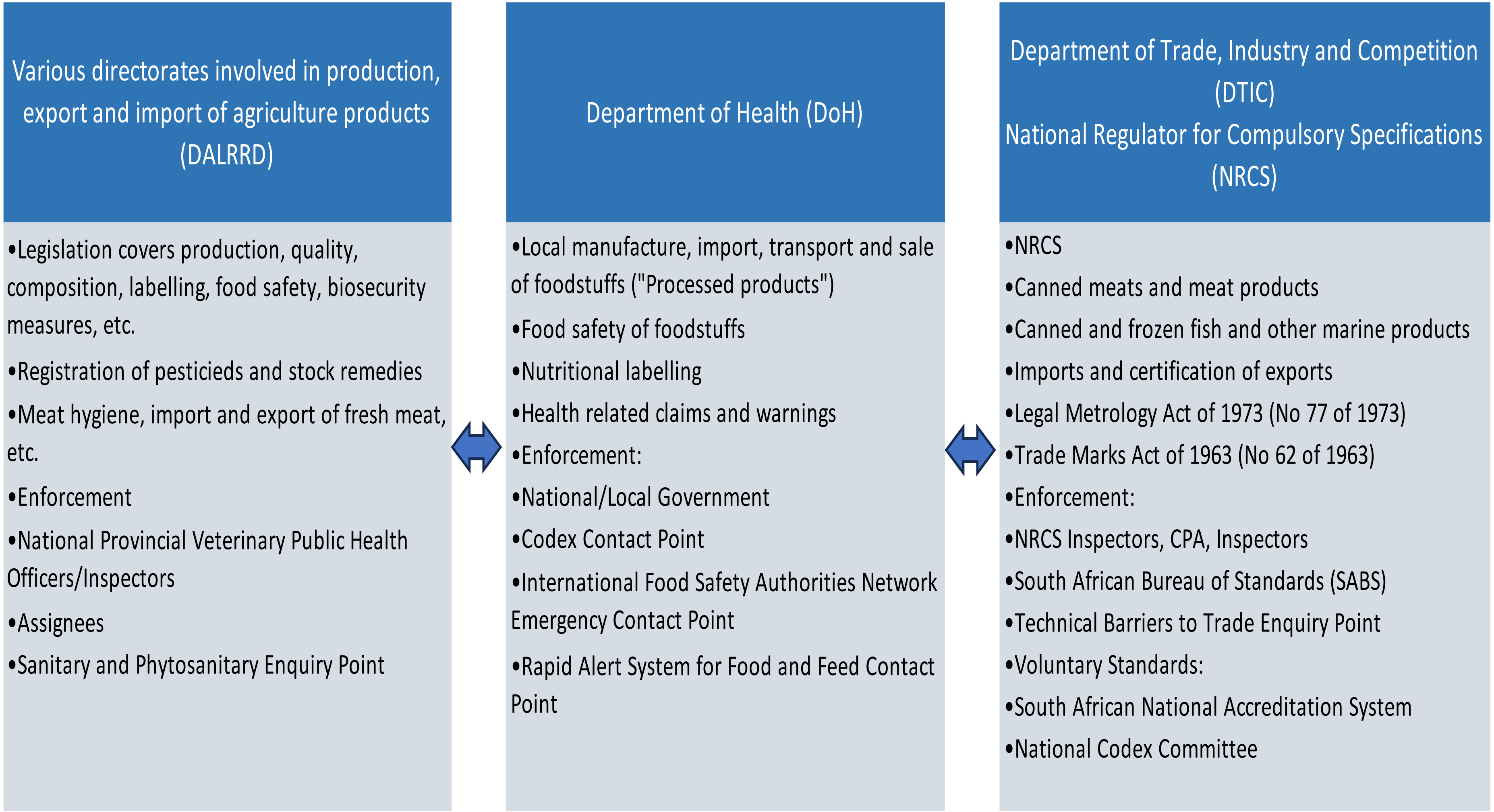
Table 5.1: List of applicable sanitary and phytosanitary legislation, (DoH, DTI, & DAFF, 2013)

Administrating by		Description of Acts		Enforced by	Summary	Key areas of compliance	Products
Department / agency	**Directorate / Cluster**	**Act**	**Act number and year of promulgation**				
DALRRD	Directorate Plant Health	Agricultural Pests Act	Act No 36 of 1983	National	Provide for measures by which agricultural pests may be prevented and combatted	• Import requirements • National control measures (pest eradication • Integrated pest management/ Good Agric Practices, Surveillance of exotic pests, etc.)	• Plants and plant products and related regulated material
DALRRD	Food Safety and Quality Assurance	Agricultural Products Standards Act	Act No 119 of 1990	National/ Perishable Produce Export Certification Agency (PPECB)	Provides control over the sale and export of certain agricultural products; control over the sale and import of certain agricultural products; and control-over other related products	• Quality standards • containers, packing and marking requirements • Sampling procedures • Methods of inspection • offences and penalties • Repeal of regulations, etc.	• Agronomic • Deciduous • Processed products (animals & plants) • Vegetables • Citrus and subtropical fruit
DALRRD	Directorate Animal Health	Animal Diseases	Act No 35 of 1984	National	Provides for the control of animal diseases and parasites and provides measures for the promotion of animal health	• National Control measures(surveillance, eradication, Vaccinations, record keeping on farm biosecurity measures, etc. • Import control measures	• Animals and animal products
DALRRD	Directorate Agricultural Inputs Control	Fertilizers, Farm Feeds, Agricultural Remedies and Stock Farm Remedies Act	Act No 36 of 1947	National	Provides for the registration of fertilizers, farm feeds, agricultural	• Safe use and handling of agricultural products • Registration of agricultural inputs	• Fertilizers • Farm Feeds • Agricultural Remedies

Administrating by		Description of Acts		Enforced by	Summary	Key areas of compliance	Products
Department / agency	Directorate / Cluster	Act	Act number and year of promulgation				
					remedies, sterilising plants and pest control operators, among others	- Import requirements, etc.	- Stock Remedies
DALRRD	Food Safety and Quality Assurance	Liquor Products Act	Act No 60 of 1989	National	Provides for the control over of the sale of certain alcoholic products	- Sale and production - Quality Standards - Labelling and marking Import and export requirements	- Wine; - A spirit; - A grape-based liquor; - A spirit-based liquor; - A specially authorized liquor
DALRRD	Veterinary Public Health	Meat Safety Act	Act No 40 of 2000	National	Provides for measures to promote meat safety and the safety of animal products; establish and maintain essential national standards in respect of abattoirs; to regulate the importation and exportation of meat	- Quality standards - Hygienic regs - Packing and marking requirements - Registration of facilities Inspections, etc.	- Meat and meat products
DoH	Medicines - Regulatory Affairs	Medicines and Related Substances Act	Act No 101 of 1965	National	Provides for the registration of medicines intended for human and for animal use	- Oversee, monitor, evaluate regulations and registrations for medicines and medical equipment	- Medicine (veterinary and for human consumption. - Medical equipment

Administrating by		Description of Acts		Enforced by	Summary	Key areas of compliance	Products
Department / agency	**Directorate / Cluster**	**Act**	**Act number and year of promulgation**				
the DTIC	NRCS	National Regulator for Compulsory Specifications Act	Act No 5 of 2008	National	Provides for the existence of the NRCS, which is responsible for Compulsory standards regarding certain forms of meat and fish	• Development, promotion and maintenance of independent food standards and quality systems. • Certification, auditing and assessment services for food and beverage processes and food product testing • Equipment, accessories, services, and standards • Serve as South Africa`s World Trade Organization / Technical Barriers to Trade Point of Contact	• Meat and fish products • Beverages and food products
DoH	Food Control	Foodstuffs, Cosmetics and Disinfectants Act	Act No 54 of 1972	Provincial/ local	Control the sale, manufacture and importation and exportation of food stuffs, cosmetics and disinfectants	• Food legislation and regulations related to food safety, food labelling, and advertisement • Audit and support Port Health Services and Municipal Health Services. • Evaluate risk assessments related to agricultural chemicals and food produced through biotechnology for DALRRD. • South Africa's National Contact Acts as Point for the joint FAO/WHO Codex Alimentarius Commission, • International Food Safety Authorities Network and the	• All export and import of food products from animal and plant origin; • Safe foodstuffs for human consumption • Cosmetics • Disinfectants

Administrating by		Description of Acts		Enforced by	Summary	Key areas of compliance	Products
Department / agency	Directorate / Cluster	Act	Act number and year of promulgation				
						European Union Rapid Alert System for Food and Feed	



Various directorates involved in production, export and import of agriculture products (DALRRD)

- Legislation covers production, quality, composition, labelling, food safety, biosecurity measures, etc.
- Registration of pesticieds and stock remedies
- Meat hygiene, import and export of fresh meat, etc.
- Enforcement
- National Provincial Veterinary Public Health Officers/Inspectors
- Assignees
- Sanitary and Phytosanitary Enquiry Point

Department of Health (DoH)

- Local manufacture, import, transport and sale of foodstuffs ("Processed products")
- Food safety of foodstuffs
- Nutritional labelling
- Health related claims and warnings
- Enforcement:
- National/Local Government
- Codex Contact Point
- International Food Safety Authorities Network Emergency Contact Point
- Rapid Alert System for Food and Feed Contact Point

Department of Trade, Industry and Competition (DTIC)
National Regulator for Compulsory Specifications (NRCS)

- NRCS
- Canned meats and meat products
- Canned and frozen fish and other marine products
- Imports and certification of exports
- Legal Metrology Act of 1973 (No 77 of 1973)
- Trade Marks Act of 1963 (No 62 of 1963)
- Enforcement:
- NRCS Inspectors, CPA, Inspectors
- South African Bureau of Standards (SABS)
- Technical Barriers to Trade Enquiry Point
- Voluntary Standards:
- South African National Accreditation System
- National Codex Committee

Figure 5.3: The various food regulators that is forming part of the South African Food Control System, (DoH, 1972; DTIC, 1973; DoH, 1977; DALRRD, 1983; DTIC, 1993; DALRRD, Genetically Modified Organisms Act, 1997; DALRRD, 2000; DTIC, Liquor Act, 2003, DTIC, National Regulator for Compulsory Specifications Act, 2008; (CRS), 2014)

In Government Notice No. R. 2336 - 19 October 1979, seven (7) colour classifications were indicated for honey. The colour is to be determined by a Pfund honey grader. The colour specifications (colour classes) were as follows: water white from 1 mm to 8 mm on Pfund scale; extra white from 9 mm to 17 mm; white from 18 mm to 34 mm; extra light amber from 35 mm to 50 mm; light amber from 51 mm to 58 mm; amber from 86 mm to 114 mm; and dark honey with more than 114 mm. To be classified as a uniform colour, less than five percent of the containers of the honey's colour may differ from the predominant colour of the total quantity of honey by more than one colour class either way. In the recent APS Act, the colour classification is not indicated as such it is only mentioned in the grading section that the honey should be uniform in colour and clear (Department of Agricultural Economics and Marketing, 1979; DALRRD, 2000).

Other amendments include the following: HMF is lowered from 80mg/kg to 40mg/kg, a uniformed moisture content (%) of not more than 20% has been set, as in the Government Notice No. R. 2336 – 19 October 1979, and the percentage moisture content is set for each grade (choice grade, standard grade and under grade) (DoH, 1972; DALRRD, 1983; DALRRD, 2000). Furthermore, grades were reduced from three to two grades, as the under grade was discarded. The only two grades in the recent APS Act are choice grade and industrial grade. Honey mixtures grades are reduced to one grade namely choice grade. The minimum percentage of honey and other ingredients in a mixture was also removed together with the °Brix readings that were specified for each grading.

The following physico-chemical parameters were added to the revised version, namely: invertase, amylase, water insoluble solids, proline and stable carbon isotope analysis. In addition, the only two acts included in the recent APS Act, are that honey and bee products should comply to the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (No 54 of 1972) and the Agricultural Pests Act, 1983 (No 36 of 1983) (DoH, 1972; DALRRD, 1983). The net mass of the container should comply to the Trade Metrology Act, 1973 (No 77 of 1973). The Health Act, 1977 (No 62 of 1977) was removed (DoH, 1972; Department of Agricultural Economics and Marketing, 1979; DALRRD, 1983; DALRRD, 2000) (Department of Agricultural Economics and Marketing, 19 October 1979) (DAFF, 1983) (DAFF, 2000). All the amendments made to the national standard (APS Act) in 2000, are in line with specific needs of the South African consumer, and with international standards such as the Codex Alimentarius Standard for honey, the International Honey Commission (IHC) and the EU Standard (Bogdanov, et al., 1999; DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002). The latest Codex standard, that was updated in 2019, still covers the same compositional requirements and testing methods specified in the previous Codex standard, as well as those in the South African regulations (Crewe, Masehela, Human, & Pirk, 2021).

5.5.2.2 Comparison of the European Directive 2001/110EU, Codex Standard for Honey and APS Act, 1990 (Act No 119 of 1990)

Although Codex forms the basis of most compiled legislations that applied to honey in the world, legislations have been amended and adapted for the honey market of each country. The EU Directive standards and Codex Alimentarius Standard for honey differ slightly from each other, as the EU Directive is less specific. By comparing the EU Directive standards and Codex Alimentarius Standard for honey with the APS Act differences were identified.

The Codex Alimentarius Standards are voluntary application for assessing the quality of honey by governments, while the EU Directive standards are all quality standards that have to be complied with by the Member States. Hence, the EU has a more stringent approach to compliance than South Africa (Bogdanov, et al., 1999; Thrasyvoulou, et al., 2018). For example, honey cannot be sold in the EU labelled as “honey” if it does not comply to the definition of honey as specified by the EU Standards (Elflein, 2015). The EU Directive standards specify that honey has to be produced by *A. mellifera* bees, while the APS Act states that honey is produced by honeybees (*Apis* genus) or stingless bees (*Melliponinae* order) (DALRRD, 2000; European Commission, 2002).

Recently, much attention has been given to honey produced from stingless bees. Current studies done on the honey of stingless bees, show that stingless bee honey differs with regards to the viscosity, colour and taste (Razali, et al., 2018; Sahlan, et al., 2019). In addition, honeydew honey, made from sweet juices of other parts of flowering plants (excretions or secretions of plant sucking insects) is characterised by higher disaccharides, trisaccharides (melezitose, raffinose), high F/G, pH-value, electron conductivity, net absorbance, ash percentage, high concentrations of free acidity, polyphenol, amino acids and proteins. Furthermore, it is darker than blossom honey with peculiar sensory features (Seraglio, et al., 2019).

According to the Codex Alimentarius Standard for honey, honey is produced by all honeybees (Codex Alimentarius, 2001). Due to this definition, supplementary information is vital to define honey made by other bee species (Thrasyvoulou, et al., 2018; European Commission, 2002). Currently, there is no official definition for honey made by other bee species in the standards used in Europe (Codex Alimentarius, 2001; European Commission, 2002). Both the EU Directive and Codex Alimentarius Standard for honey describe plant sucking insects as members of the Hemiptera order that produces honeydew. The APS Act is, however, not as specific. The APS Act, states that honeydew is dextrorotary. In South Africa, honey is deemed as non-complaint to the standards for grading if it does not adhere to the definition of honey and the prescribed standards for the composition, quality or ripeness of honey as specified by the APS Act. In contrast to the APS Act the Codex Alimentarius Standard for honey and EU Directive prescribe the methods to be used for honey sample preparation for the assessment of quality parameters (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002).

Both the Codex Alimentarius Standard for honey and the APS Act, have a section dealing with hygiene, contamination and sugar adulteration. The Codex Alimentarius Standard for honey mentions hygiene, metals, residue of pesticides and veterinary drugs under Contaminants (DALRRD, 2000; Codex Alimentarius, 2001). In the APS Act, the hygiene and contamination of honey are dealt with in the Foodstuffs, Cosmetics and Disinfectants Act 1972 (No 54 of 1972) and the Agricultural Pests Act, 1983 (No 36 of 1983) (DoH, 1972; DALRRD, 1983). It is also mentioned in all three standards that honey should be well ripened (minimum moisture content, maximum enzyme activity and sealed in cells) and be free from any foreign particles in its composition e.g., mould, insects or any insect fragments, etc. Honey should not have any foreign taste, odours, signs of fermentation and not be treated in such a way that the chemical composition is compromised (DALRRD, 1983; DALRRD, 2000).

The Codex Alimentarius Standard for honey states that “honey shall not have added to it any food ingredient, including food additives, nor shall any other additions be made other than honey. Honey shall not have any objectionable matter, flavour, aroma, or taint absorbed from foreign matter during its processing and storage, honey shall not have begun to ferment or effervesce. No pollen or constituent particular to honey may be removed except where this is unavoidable in the removal of foreign inorganic or organic matter. Honey shall not be heated or processed to such extent that its essential composition is changed and/or its quality is impaired. Chemical or biochemical treatments shall not be used to influence honey crystallisation” (Codex Alimentarius, 2001). This statement implies honey should be made by honeybees and not by human involvement (APIMONDIA Working Group, 2019). In addition, it is also intended to address the use of vacuum drying to artificially dehydrate honey instead of relying on the natural maturation process (Rust, 2020). The EU Directive shares the same specifications, additionally stating that honey, with the exception of baker’s honey, must not have an artificially changed acidity or have been heated in such a way that the natural enzymes have either been destroyed or significantly inactivated (European Commission, 2002). The EU Directive has a section that defines baker’s and industrial honey, as well as honey with a natural low enzyme content. Honey is classified as a baker’s honey if it has a foreign taste or odour, or has been fermented, has begun to ferment and has been overheated (Thrasyvoulou, et al., 2018).

Baker’s honey is suitable for industrial uses or as an ingredient of other foodstuffs (European Commission, 2002). In neither the Codex Alimentarius Standard for honey, nor the APS Act, is baker’s honey defined. Consequently, a baker’s honey definition would be interpreted to be included under

Industrial Grade Honey in the APS Act (DALRRD, 2000; Codex Alimentarius, 2001). In addition, the APS Act, stipulates that honey should be free from impurities, additives, and adulterants and that it shall not have any foreign taste, odours, begin to ferment and not be treated in such a way that the chemical composition is compromised, for example, destroying or inactivating enzymes (DALRRD, 1983; DALRRD, 2000). Another difference between these three standards is that the EU Directive and Codex Standard for honey make provision for various expectations where honey from certain plants and climates differs from the standard composition, while the SA regulations are rigid and do not allow for any exceptions (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002). As seen in Table 5.2, honey is a biological product and its composition can vary due to various factors, such as climatic conditions, floral sources which provide nectar and pollen, geographical origin, etc. (Chin & Sowndhararajan, 2020).

The APS Act is more specific regarding the requirements for honey and mixtures of honeybee products. It has a section relating to the grading of honey, including choice and industrial grading. Liquid honey, comb and creamed honey can be classified into both grades, but chunk honey and mixtures of bee products have only one grade, choice grade - while raw honey has no grading except if filtered. The honey grading should be indicated on the label, however, the analysis required for grading is usually not performed and the grade of the honey is not included on the label (DALRRD, 2000). Both the EU Directive and Codex Alimentarius Standard for honey only indicate the modes of production and styles on the label (Codex Alimentarius, 2001; Codex Alimentarius, 2001) (Codex Alimentarius, 2001; European Commission, 2002). It was agreed that the SA regulations should be updated to reflect the extraction methods on the labelling when the APS Act is revised (Crewe, Masehela, Human, & Pirk, 2021).

In Table 5.2, additional assessments for compositional characterisation, which are not mentioned in the EU Directive and Codex Alimentarius Standard for honey, are indicated in the APS Act (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002). The additional criteria, which fall outside the legal requirements, includes additional regulations regarding quality related to reducing sugars, total acidity (free acid + lactones), protein testing (Lund-test), invertase activity, direct and immediate specific rotation, fructose/glucose (F/G) ratio, density, ash, amylo-and erythrodextrine content, proline content, amylase, invertase, and stable Isotope ratio ^{13}C (Bogdanov, et al., 1999). The standards indicated in the APS Act, do not make provision for exceptions compared to the EU Directive and Codex Alimentarius Standards for honey regarding various physicochemical parameters (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002). Specifications that have been legislated by other countries that are mentioned in the APS Act, include invertase activity and F/G ratio (Germany and Turkey), ash content (Argentina, Canada, China and India), starch and dextrine

(Argentina and Japan) and proline (Poland and Turkey). In addition, Turkey also legislated the difference between the protein and honey delta ^{13}C and ^{4}C sugars ratio, as well as the maximum limit for naphthalene, while Japan has set limits for heavy metals (Thrasyvoulou, et al., 2018).

Traceability of the origin of honey is indicated in all three standards. An indication of the origin as well as the necessary documentation has to accompany the honey product. The Codex Alimentarius Standard for honey requires the indication of the producing country on the label. Similarly, the EU Directive states that the country or countries of origin where the honey has been harvested shall be indicated. However, in the EU Directive, if honey originates from more than one member state or from a third country, that indication may be replaced with the words *"blend of EU honey", "blend of non-EU honey"*, or "*blend of EU and non-EU honey"*. This provision is not valid for in the Codex Alimentarius Standard for honey (Codex Alimentarius, 2001; European Commission, 2002).

In the APS Act, labelling of the country of origin is also a requirement. Labels for blended honey can be cryptic when indicating the country of origin, for example: indicating "South Africa and/or Argentina/China/Uruguay/Zambia/Poland". Traceability of honey is prescribed in the regulations and can also be used as a method to prevent adulterated honey from being sold since adulterated honey will not be fully traceable to the premises where the product was produced/packed (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002). The standards and regulations were established to prevent adulteration and mislabeling of honey and to protect its safety and quality. However, there are discrepancies between the standards and regulations in the type of honey and its physico-chemical parameters due to different beekeeping conditions in different countries. Legislations cannot always make provision for the complexity of honey variations, so there are cases where the physico-chemical parameters of authentic and unprocessed honey fail to comply with the composition criteria of regulations. This results in financial losses for beekeepers when attempting to distribute their products (Thrasyvoulou, et al., 2018).

5.5.3 The role physico-chemical parameters play in determining the quality of honey

Quality in honey generally refers to its genuineness and natural quality, and the absence of adulteration, residues, damage from heat and storage and other unwanted qualities. Quality control measures in honey production limit or ban the presence of residues from antibiotics and pesticides, minimize the levels of HMF, set limits for various physico-chemical parameters for example moisture content, diastase, pollen, sugars, acidity and amino acid profiles and define the required sensory values (DALRRD, 2000).

Monitoring the quality of honey is important for the honey trade as honey laundering is increasing due to a declining production rate. Because South Africa has become a net importer of honey, it has also become a prey for economically motivated adulteration. Masking the botanical and geographical origin is a form of honey laundering. Honey laundering involves the process where honey is filtered to remove pollen or soil that could be used to trace it back to its origin (Guelpa, Marini, du Plessis, Slabbert, & Manley, 2017; Thrasyvoulou, et al., 2018). For example, the botanical and geographical origin are masked by using ultra-filtration in the Chinese honey market resulting on no color or flavor. In 2011 Chinese honey was shipped to India and Thailand where it was re-labelled before entering the EU (Isopescu, Josceanu, Colta, & Spulber, 2017; Eshete, 2019a). Masking the geographical origin of honey is a contradiction to the EU's 1169/2011/EU and 37/2010 EU regulations which state that the indication of production origin is obligatory (EU, 2010; EU, 2011).

The focus of physico-chemical parameters is to determine the botanical and geographical origin as well as to distinguish between pure and adulterated honey (Warui, et al., 2019). Origin identification of honey is a complex task as legislation for compositional standards of physico-chemical parameters is not available, nor prescribed in the Codex Alimentarius Standards for honey, the EU Directive or the APS Act, specifically for monofloral honey. Without legislating the characteristics, it is not possible to correctly identify the origin of monofloral honey. There are several variables to consider related to monofloral honey production, such as local flora, geography (location), soil or climate, and corresponding volatile analysis, including compound isolation and analytical procedures. Examples of "presumed monofloral honey" in South Africa include sunflower, canola, aloe, citrus blossom, macadamia, litchi, a diversity of Eucalypts, as well as Protea, Strandveld and Sandveld fynbos (Rust, 2020). Only certain countries, for example Germany, Greece, Serbia, and Turkey, have legislations for monofloral honey. Turkey has a set of physico-chemical characteristics for almost all monofloral honey produced in its country (Thrasyvoulou, et al., 2018).

Botanical origin influences the price of honey. Monofloral honey is considered to be of a higher quality than polyfloral honey due to its distinctive flavour and taste (Guelpa, Marini, du Plessis, Slabbert, & Manley, 2017; Bodor, et al., 2020). Traditionally, melissopalynology is used to determine the floral and geographical origins of honey, however it is not prescribed in the APS Act, as a physico-chemical characteristic, but only mentioned as part of grading (DALRRD, 2000). This is also a useful screening method for detecting cane sugar adulteration, but it is currently inadequate for detecting synthetically prepared syrups, to detect added pollen or inadequate filtration (Soares, Amaral, Oliveira, & Mafra, 2017; Tura & Seboka, 2019). Furthermore, optical rotation distinguishes types of nectar honey (usually with negative values) from types of honeydew honey (with positive values) (Jakubik, Borawska, & Socha, 2020). Other physico-chemical parameters, such as proline and electron activity, are also considered important in determining the botanical origin (Czipa, Phillips, & Kovacs, 2019).

Sensory analysis is a classical method that is used to asses quality, evaluate conformity to establish the sensory profiles specifically of monofloral honey, verify the absence of taints and to understand consumer preference for honey (Milojković Opsenica, Lušić, & Tešić, 2015; Marcazzan, Mucignat-Caretta, Marchese, & Piana, 2018). Sensory analysis is not prescribed in the APS Act but is mentioned in the requirements for grading. As for the taste of liquid, creamed, comb and chunk honey this quality factor has to comply with the requirements for choice grade even if it is industrial grade. The taste should be typical of the predominant source/s from which it is derived. In addition, it should be well ripened and free from any foreign aroma or flavour caused by processes such as overheating or smoking, and it should not have an unnatural colour. To determine if grading has been correctly done, the sensory test will, therefore, have to be added to the array of quality parameters (DALRRD, 2000). However, there is no requirement nor standard in the APS Act, to test these characteristics. It is, therefore, recommended that the International Organization for Standards (ISO) standards for evaluating sensory defects and conformity of monofloral honeys should be used as guidelines, or the ISO standards can be incorporated into the APS Act (Marcazzan, Mucignat-Caretta, Marchese, & Piana, 2018; DALRRD, 2000).

In a study on Ecuadorian commercial honey, honey made by *A. mellifera* had a characteristic floral taste, whereas artificial honey had a candy like odour-aroma (Schievano, Zuccato, Finotello, & Vit, 2015). Botanical origin, and geographical and seasonal conditions are all factors that influence the taste of honey (Kružik, Grégrová, Rajchl, & Čížková, 2017). Detection of adulteration, mislabeling and contamination is possible. Adulteration with sugar, sugar solution or products with the same composition and consistency as honey e.g., the addition of high-sugar corn syrups or sugar-based adulterants and dilution of honey with water, can all be detected (Sammataro & Weiss, 2013; Prajapati, Agarwal, & Manju, 2019).

Colour, together with aroma and taste, is one of the sensory parameters that attracts consumers to purchase and consume honey (Karabagias, 2017; Halagarda, Groth, Popek, Rohn, & Pedan, 2020). This physico-chemical parameter is not specified in the APS Act, as a characteristic that must be determined but as part of the grading requirements. For the grading of choice and industrial grades of liquid and creamed honey, their colours should be uniform and clear, but for creamed the colour should be uniform and creamy. Raw honey does not have a colour grading, but if honey is processed, then it should comply with the requirements and specifications of choice grade liquid honey. Raw honey is classified as "unfiltered” and “unheated honey”. Unheated honey is honey of which the temperature has not been increased above 38°C by the producer/beekeeper (DALRRD, 2000). Colour plays an important role in consumer preference. The colour of honey varies between light to black amber tones and it reflects the antioxidant properties, nectar source, pollen content, mineral content, storage time, flavonoid content and temperature of honey in the hive (Karabagias & Karabournioti, 2018; Sharma, Vaidya, Kaushal, & Singh, 2020). Adulterated honey appears brighter, while pure honey is more reddish (Chua & Hamzah, 2020). The flavour and aroma usually vary due to plant origin, as can be ascribed to its relation to volatile compounds (Machado De-Melo, Almeida-Muradian, Sancho, & Pascual-Maté, 2018).

It is evident that various factors contribute to honey’s compositional differences e.g., maturity, purity, degree of deterioration and adulterants. Physico-chemical analysis to evaluate the quality of honey is divided into the following groups, namely 1) maturity, which includes analysis of reducing sugars, moisture, apparent sugars; 2) degree of deterioration which includes analysis of free acidity, diastase activity, HMF; 3) purity which entails analysis of solids insoluble in water, minerals, ash; and finally 4) adulterant assessment which includes the Legol, Lund and Fiehe Tests (Salazar, Freitas, de Luz, & da Bersch, 2017). The quality of honey is also determined by its taste, consistency, and aroma. These are the most important parameters that affect consumer acceptability of the product (Karabagias & Karabournioti, 2018; Tsagkaris, et al., 2021).

In summary, honey must meet certain qualitative and quantitative criteria to be considered as a high-quality product (Warui, et al., 2019). However, although these physico-chemical parameters are valid all over the world, they do not satisfy all the set standards of all countries. Consequently, regular re-examination of trends in honey laundering is important to ensure honey authentication.

5.5.4 The role methodology plays in authentication of honey

It is a great challenge to ensure that the methods used in monitoring the quality of honey are able to address the more sophisticated adulteration processes used nowadays. Many methodologies are used to establish honey authentication (origins) and to monitor quality control. However, with no standardised methods to determine the quality of honey, it is best to choose methods tailored to each specific situation due to the nature of honey fraud (Marquele-Oliveira, et al., 2017). Detection of adulterated honey is mostly focused on the sugar profile (Chua & Hamzah, 2020). Different analytical techniques are being applied to detect adulteration. Each of these has its own advantages and limitations (Chua & Hamzah, 2020). Physico-chemical analysis is used to monitor the botanical origin of honey. Consequently, the honey physico-chemical characteristic determination is mostly applied, as it requires minimal instrumentation and the cost of such an analysis is low in comparison to instrumental techniques (Tsagkaris, et al., 2021).

Chromatographic techniques are mostly used to determine the honey’s botanical source based on sugar, amino acid, phenolic and flavonoid profiles. Molecular techniques are used to determine the floral and entomological honey source/s, using specific DNA markers, which are characteristic of the bee species. Elemental profiles are good indicators of geographical origin. The geographical and botanical origin of honey can be determined with the aid of isotopic techniques which are used in combination with other techniques (e.g., elemental or chromatographic). The stable carbon isotope ratio analysis (SCIRA) is the official method for the detection of honey adulteration. It is based on the stable carbon isotope ratio, $^{13}C/^{12}C$ and it also indicates the addition of C_4 sugars.

Vibrational and fluorescence techniques are used to detect adulteration, as well as botanical origin. Spectroscopic methods are very informative and widely used for both quantitative and qualitative analyses. The methods differ with respect to: 1) the species to be analysed (such as molecular or atomic spectroscopy); 2) the type of radiation-matter interaction to be monitored (such as absorption, emission, or diffraction); and 3) the region of the electromagnetic spectrum used in the analysis (Chin & Sowndhararajan, 2020; Tsagkaris, et al., 2021). Spectroscopic techniques (e.g., infrared, spectroscopy, Raman spectroscopy) are used to determine the botanical, location of production (geographical) or entomological origin requiring minimal sample preparation. In addition, adulterants have been determined with these techniques (Chin & Sowndhararajan, 2020).

Nuclear Magnetic Resonance spectroscopy (NMR) has simple to no sample preparation, high sensitivity and reproducibility, but it is costly, due to the complexity of the analysis, maintenance and skilled operators that are needed. This is a fast-screening technique but needs to be continuously improved by database extension and harmonisation. However, confirmation of non-conform results by other methods is necessary, as a high number and variety of samples are necessary to build reliable spectral databases (Tsagkaris, et al., 2021). Pollen analysis or melissopalynology, is the traditional method of verifying botanical origin (Jakubik, Borawska, & Socha, 2020).

Currently, methods for honey monitoring are based on rather expensive analytical instrumentation and skilled operators, as well as tedious analysis which each have their own limitations of detection limits and detection of adulterants. As there is no rapid, sensitive, inexpensive and accurate technique available on the market to monitor the quality of honey, the development and evaluation of new methods are justified (Koncz, et al., 2017). The IHC was created to compile new honey standards and methods to address the strict market requirements for honey (Marquele-Oliveira, et al., 2017). Current market requirements include low-cost methods that are faster and are characterised by the highest accuracy and precision (Jakubik, Borawska, & Socha, 2020).

Furthermore, screening methods in conjunction with chemometric analysis have become more important. Chemometric methods are used to analyse spectral information and generate mathematical models to identify honey fraud. A few examples of statistical methods are principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), linear discriminant analysis (DLA), etc. (Mendez, Mendoza, Cruz-Tirado, Quevedo, & Siche, 2019; Tsagkaris, et al., 2021). However, the use of these simple and fast methods encounters difficulties in differentiating between different honeys (monofloral and polyfloral) and their blends. Consequently, combination with other methods, such as sensory analysis, may assist in authentication (Koncz, et al., 2017). As trained and experienced individuals are needed for sensory analysis, and this method has an inherent lack of standardisation, sensory analysis has limitations. To counter these limitations, the so-called electronic tongues (e-tongues) or electric noses (e-nose) have been developed (Tsagkaris, et al., 2021).

Overall, it is vital to monitor and control the origins of the honey and this is only feasible with the combination of reliable analytical methods and advanced chemometric tools. Because no single method is available to ensure authenticity and purity of honey, this combination of testing methods significantly reduces the risk of inappropriate sugar addition and identifies previously tailor-made and unrecognised sugar syrups being used for honey adulteration (Tsagkaris, et al., 2021). Consequently, the standards and the methods applied should be adjusted in conjunction with emerging trends by developing new technologies and adapting already established technologies.

5.5.5 Standard and technique requirements perceived as trade barriers

Stringent quality requirements and the enforcement of these legislations create entry barriers to foreign products which contribute to honey laundering. Importing countries impose sanitary and phytosanitary measures which are laws, rules, standards and procedures that governments employ for environmental and health concerns. Sanitary regulations apply to animal-based products (i.e., meats, poultry and dairy products) to ensure that they meet or exceed specified sanitary standards. In addition, these regulations also apply to fruits, vegetables, bulk commodities, and other plant-based products (Table 5.1).

Technical barriers to trade (TBT) include technical regulations, product standards, environmental regulations and voluntary procedures relating to human health and animal welfare. Other TBTs include related health and quality standards, testing, registration and certification requirements; safety and industrial standards and regulations; conformity assessments and packaging and labelling regulations, including trademarks; quarantines; and advertising and media regulations. These multilateral trade rules allow governments to apply sanitary and phytosanitary measures for the protection of the life and health of humans, animals and plants. These measures should not arbitrarily or unjustifiably discriminate between countries where similar conditions prevail or act as a disguised restriction to international trade ((CRS), 2014).

An example of a technical barrier to trade is where the EU defines honey as a product only of the European honeybee, *A. mellifera*, thus preventing the sale to the EU of honey produced by Asian

Table 5.2: Summarised comparison of the compositional criteria for honey in the European Directive, Codex Alimentarius Standard and the Agricultural Product Standards Act, (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002)

Compositional criteria	**European Directive 2001/110 EU**			**Revised Codex Standard 2001**		**APS Act,1990 (Act No 119 of 1990)**
	Blossom honey	Blossom Honey Exceptions	Honeydew Honey		Exceptions	No indication for baker's honey in Act
Moisture (%)	≤20	*Calluna* and baker's honey <23 Baker's honey from *Calluna* <25	≤20	All honey ≤20	*Calluna* ≤23 No indication for baker's honey	All honey ≤20
Sum of fructose and glucose (%)	≥60	-	≥45	Blossom honey ≥60 Honeydew honey and blends of honeydew with blossom honey≥45		
Sucrose (%)	≤5	*Robinia, Medicago, Banksia, Hedysarum, Eucalyptus, Eucryphia* spp, and *Citrus* spp <10 *Lavender (Lavandula spp)* and *Borago* (*Borago officinalis*) <15	≤5	All honey ≤5	*Robinia, Medicago, Banksia, Hedysarum, Eucalyptus, Eucryphia* spp, and *Citrus* spp <10 *Lavender (Lavandula spp)* and *Borago* (*Borago officinalis*) <15	All honey ≤5
Water insoluble (%)	≤0.1	Pressed honey ≤0.5	≤0.1	Honey other than pressed honey ≤0.1	Pressed honey ≤0.5	All honey ≤0.1
Electrical conductivity mS.cm^{-1}	≤0.8	*Chestnut*, *Arbutus*, *Erica*, *Eucalyptus*, *Tilia*, *Calluna*, *Manuka* and *Melaleuca*	≥0.8	Honeydew and chestnut honey and blends of these ≥0.8	*Chestnut*, *Arbutus*, *Erica*, *Eucalyptus*, *Tilia*, *Calluna*, *Manuka* and *Melaleuca* ≤0.8	
Free acid (meq/kg)	≤50	Baker's honey ≤80	≤50	≤50 all honey		Measured as total acidity (Sum of free acid and lactone) ≤40
Diastase activity (DN)** (Schade scale)	≥8	Baker's honey and honey with low natural enzyme content: >3 when HMF is less than 15 mg/kg	≥8	All honey ≥8 Honeys with low natural enzyme content: >3		≥4 DN (Göthescale)
Hydroxy-methyl-furfural (HMF) (mg/kg)**	≤40	Baker's honey, honey of tropical climate and blends of these types of honey ≤80	≤40	≤40 all honey types	Honey of tropical climate and blends of these types of honey ≤80	All honey types ≤40

Invertase (ppm)						All honey types ≥4 ppm
Proline (mg/kg)						At least 200 mg/kg
Direct and Immediate Specific Rotation						An aqueous solution containing 26 g of floral honey in a total volume of 100ml shall be not less laevorotatory than -10 degrees at 20°C
Reducing Sugar (g/100g) (Sum of Fructose Glucose and Maltose)						Flowers ≥65 Honeydew ≥60
Fructose/Glucose ratio						Should not be less than 1.0:1
Ash (g/100g)						≤0.6
Lund (cm^3)						≥0.6
Amylase (ppm)						≥8
Amylo-and erythrodextrine						Test for their presence shall be negative
Density (g)						≥1.40875 g at 20°C
Stable Isotope ratio ^{13}C (‰)				Method prescribed as part of authenticity, but no standard		Difference between the isotope ratio delta of the honey and the stable isotope ratio of its protein content shall not be less than 0.0

*Honeydew honey and blends of honeydew honey with blossom honey

**Determined after processing and blending

Countries (European Commission, 2002). This is viewed by exporting countries as a violation of World Trade Organization (WTO) provisions that require countries to treat imports and domestic (local) products equivalently and not to favour products from one source over another (Partap, Gurung, & Joshu, 2012). In this regard, the WTOs Agreement of the Application of Sanitary and Phytosanitary Measures (WTO, 1995a) and its Agreement on Technical Barriers to Trade (WTO, 1995b) recognise the right of all export and import countries to reject the importation of unsafe products; however, scientific justification for rejection should be provided (WTO, 1995a; WTO, 1995b). The WTO Agreement of Technical Barriers to Trade (WTO TBT), to which South Africa is a signatory, requires that standards and technical regulations should not be used as barriers to trade. National standards should be aligned with, or be identical to, recognised international equivalents, and technical regulations should comply with international regulatory norms. Similar requirements apply to the WTO Agreement on Sanitary and Phytosanitary Measures (WTO SPS, the agreement covering food), and to Legal Metrology (i.e., measurements and measuring instruments used in trade incl. legal, health, safety and environment) (Mukumba, 2011).

The imposed sanitary and phytosanitary measures for environmental and health concerns and technical rules on product definitions, essential composition, packaging, labelling, and other factors are in line with our policies and legislation. Improper use of SPS and Technical Barriers to Trade (TBT) measures can create substantial barriers to trade when they amount to disguised protectionist barriers, are not supported by science or are unwarranted.

Nowadays the South African honey market is overshadowed by the international demand for honey, which is close to the point where demand cannot be met by global production. This situation opens the door to “artificial honey” or honey replacement products on the market (Crewe, Masehela, Human, & Pirk, 2021). Creating another trade barrier according to the definition of honey from the three legislations is a genetically modified honey product. A team of students from the Technion-Israel Institute of Technology developed a method to produce synthetic honey using the bacterium *Bacillus subtilis,* after genetic manipulation and reprogramming. The bacterium had been manipulated to imitate the same processes and create the same enzymes that occur in a bee’s belly as it produces honey. The product looked and smelled like honey. *Bacillus subtilis* was used because it is known to be safe, and as it is commonly found in the upper layers of soil. Evidence indicates a possible presence of this bacterium in the gut of humans and some animals as well. This artificial honey product is considered a vegan product and might be a possible solution for the declining honey production. If this product is confirmed to be safe and the taste and smell have the properties of honey, the definition of honey will have to be re-defined (George & George, 2022).

Organic honey standards are another example of trade barriers - by complying with one set of organic standards, exclusion of other markets might be endured because of countries that have different organic standards. Consequently, although organic standards share many common aspects, they vary from country to country (importers and exporters) and between certifying agencies. Hence, the main purpose of organic certification is to assure quality and prevent fraud as most organic certification is carried out by a third-party certification agency (Tung, 2018). Organic honey is characterised by being produced from natural sources and it is believed to promote environmental quality, human health and animal welfare, while also being free of contaminants (e.g., heavy metals, radioactive isotopes, organic pollutants, pesticides and veterinary drugs, pathogenic bacteria) and genetically modified organisms (GMOs) (Gomes, Feás, Iglesias, & Estevinho, 2011; Soares, Amaral, Oliveira, & Mafra, 2017). The claim that a type of honey is “organic”, is difficult to achieve, as bees forage over a surrounding area of approximately 12.6 km^2 and bees do not distinguish between conventional plants or GMO plants. To produce and to be certified as “organic honey”, the whole area in which the honey is produced, has to be organic (Soares, Amaral, Oliveira, & Mafra, 2017).

5.5.6 Genetically modified (GMOs)

Genetically modified products produced from GMOs are incompatible with the definition of organic production and consumer perception of organic products according to the Council Regulation (EC) No. 834/2007. Hence, honey produced from genetically modified organisms or derivates are not considered organic (Tung, 2018). Consequently, honey should be tested for GMOs if labelled “organic”, especially if produced in countries where GMO crops (maize, soybean, rapeseed) are grown. In South Africa, the DoH is responsible for the evaluation of GMOs (DoH, 1972). The Directive 2014/63/EU relating to honey is accordingly written to the specific labelling requirements of Regulations (EC) No 1823/2003. Consequently, it is not necessary to indicate the presence of genetically modified pollen in the labels for honey if the genetically modified pollen is not more than 0.9% of the total honey and its presence is unavoidable (Soares, Amaral, Oliveira, & Mafra, 2017).

Africa is the smallest producer of organic products, leading to a lack of information about the characterisation of certified organic honey in Africa, as well as South Africa (Tung, 2018). Nonetheless, different voluntary private standards for sustainable agricultural systems exist and the private sector is working towards the certification of organic claims in Africa (Tung, 2018). Zambia is well known for its organic honeys and is self-sustainable. This honey is certified as organic based on the UK Soil Association standards, it complies with the EU’s requirements for organic products and has a fair-trade certification from Germany (Nyau, Mwanza, & Moonga, 2013). South Africa’s government has not an approved certification system for organic products, or a specific national policy or regulation with governing in regard to the local organic food market. The domestic voluntary standard applied by the

South African Organic Sector Organization (SAOSO) is currently not recognised by international certification bodies, and government has not adopted it as a mandatory organic standard in South Africa (SAOSA, 2020). Consequently, certification and inspection of these products which claim to be organic are carried out by private certification bodies (Hendricks, 2021). However, in South Africa all organic food products must comply to the general food regulation stipulated by the Food Control Division of the Ministry of Health and the DALRRD regulations as set out in the APS Act (DoH, 1972; DALRRD, 2000). However, the threshold of tolerance of non-organic material and which ingredients and substances can be used in organic farming is not indicated. In the case of organic beekeeping, products are required to follow the rules of organic production for at least one year before they may be labelled “organic”, the hives are required to be at least six kilometers from each other and disease prevention based on stress-free practices encouraging resistance to disease and the prevention of infections must be adhered to (Tung, 2016). No foreign additives, nor objects, should be added to the honey (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002).

5.5.7 Maximum Residual Limit (MRL)

Pesticides (i.e., Neonicotinoid) and antibiotics (i.e., Chloromycetin) also become technology barriers to international trade (Commission Regulation, 2010). The maximum residual limit (MRL) of chloromycetin is another important honey safety standard which is of concern to importing countries. Chloromycetin is a bacteriostatic antimicrobial and it is effectively used for the treatment and prevention of bee diseases. It is, however, also associated with bone marrow toxicity in humans. Hence, most importing countries have a set MRL for chloromycetin limits in honey to protect human health (Wei, Huang, & Yang, 2012). Various countries such as the USA, China, Brazil and the European Union (Reg. 2010/37/UE) banned the use of chloromycetin in honey. The European Commission also set the minimum Requirement Performance Limit (MRPL) at 0.3 g/kg (Commission Regulation, 2010). In South Africa, MRL evaluation is in done by the DoH (DoH, 1972). MRL regulatory harmonisation is a complex, technical problem, with multiple contributory factors affecting most aspects of agri-food production and trade. This barrier to market access generally arises because either a country’s domestic MRL is less strict than that of the foreign MRL, or because the MRL has not been established in the specific foreign market. These rejections are becoming sufficiently frequent so as to act as major trade barriers, thus impeding the global movement of agri-food products.

5.5.8 Heating and Filtering

“No substance or matter is allowed to be removed in such a way that it changes the characteristics of the honey” (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002). In all three of the standards (the EU Directive, the Codex Alimentarius Standard for honey and the APS Act the removal of pollen is allowed via heating and filtering to improve the clarity of honey. Such pollen removal influences the classification process to determine authenticity. In ordinary circumstances, beekeepers typically strain or coarse-filter honey to remove natural physical contaminants without removing pollen (i.e., bee parts and wax) (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002). However, micro-filtering uses much finer screens, sometimes in conjunction with heating, to remove all the pollen in honey. Pollen provides important flavour and nutritional characteristics to honey and can be used to identify its origin and floral source. As described before, such 'honey laundering' prevents the identification of honey's origin based on its pollen content (Ferrier, 2021). Consequently, in this case honey should be labelled as "filtered". In South Africa, the APS Act, prescribes the sieve size (35 mesh) for removal of impurities. The APS Act specifies that botanical origin is not allowed to be indicated if pollen has been removed (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002). Honey should not be strained with a mesh size smaller than 0.2mm as to prevent the removal of pollen (Bogdanov & Gallman, 2008).

5.5.9 Other bee products

Currently the honey trade is being regulated by a small number of internationals standards that are based on the Codex Alimentarius. No standards are available for the other bee products such as beeswax, royal jelly, propolis and pollen in the APS Act (DALRRD, 2000). A suggestion for the composition criteria for royal jelly, for raw poplar propolis and polar propolis, and for pollen, were proposed by the IHC in 2007. However, the proposed standards and methods that are used in the different countries are based on standards and methods used in the industry and specifications of these countries. The extraction and production of bee products are not regulated and are based on individual processes. Standardised methods to establish these standards will be next step in legislation governing the regulation of other bee products (Bogdanov & Gallman, 2008).

5.5.10 Bee product names

The bee product names are similar in all three standards, except for baker's honey as specified by the EU's Directive (Bogdanov, et al., 1999). All three standards have the same prescription for labelling honey according to botanical origin. If honey is made predominantly from specific plant source, then it can be labelled according to the plant origin (e.g., eucalypts honey) as long as the distinctive characteristics of that specific botanical source are present. Filter and baker's honey do not have any supplementary guidelines regarding floral origin (Thrasyvoulou, et al., 2018; Codex Alimentarius, 2001; European Commission, 2002) (DAFF, 2000). Other than the EU Directive and Codex Alimentarius Standard for honey, the APS Act, specifies that there should be an indication if a honey mixture contains sugar cane honey (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002). If this is the case, it should be labelled as "containing sugar cane honey".

The terms "raw" and "unprocessed" should be used if honey contains raw honey. In the APS Act, all honey substitutes are excluded from these specified regulations except for the inclusion of specific requirements on the labels. Honey substitutes are defined as "*any food consisting of a food substance or a mixture of substances that have been made to resemble honey*" (DALRRD, 2000). The regulation stipulates that a honey substitute may not indicate the word "honey" on the label in the case of the ingredient list when honey is a component of the substitute. A honey substitute is regarded as a product that resembles honey, but contains less than 50% honey. An example of a honey substitute is *"honey flavoured syrup"* because it has a content of less than 50% honey. Another example includes using the words that resemble *"honey"*. Consequently, the labelling of honey products should be clear to ensure that it does not mislead consumers with regards to its content (DALRRD, 2000; Crewe, Masehela, Human, & Pirk, 2021). According to the EU Directive, if honey is classified as baker's honey the following must appear on the label *"intended for cooking only"* next to the product name.

If baker's honey is used as an ingredient in a composite foodstuff, it should be indicated in the list of ingredients as baker's honey (European Commission, 2002). The Codex Alimentarius Standard for honey and EU Directive prescribe the methods to be used for honey sample preparation for the assessment of quality parameters, however the APS Act, does not prescribe the methods (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002).

5.5.11 Inspection and handling

One section of the APS Act regulates the inspection and handling of honey samples prior to analyses. It specifies the procedure the inspector must follow at the time of inspection, sample analyses, as well as the method of sample preparation. The sample preparation can either be for sensitive or non-sensitive tests, depending if the honey is crystallised. For heat sensitive tests, a sample should be prepared without heating. If a honey sample is in a liquid state, it should be mixed thoroughly by stirring. If the honey is still in the comb, it should be separated from it by crushing the comb and straining the honey through a sieve of 35 mesh. For non-heat sensitive tests, the following procedure should be followed: 1) if in liquid form, mix thoroughly by stirring; 2) if honey is crystallised, it should be placed in a closed container in a water bath and heated for 30 min at 60°C while stirring occasionally. If required, it should be heated to 65°C until it has liquefied. After heating, it should be mixed thoroughly and rapidly cooled to room temperature as soon as the sample liquefies; 3) if still in the comb, it should be separated from the comb by compressing the comb and straining the honey through a sieve of 35 mesh. If combs and beeswax particles pass through the sieve, the samples should be heated to 40°C and strained through a cheese cloth in a hot water funnel. If the honey is granulated/crystallised in the comb, it should be heated to 65°C until the beeswax has liquefied. It should then be cooled followed by removal of the wax (DALRRD, 2000).

All imported honey should be irradiated according to the DoH and DALRRD regulations, but because of the changes that have been made to the characteristics of organic honey, organic honey is not irradiated (DoH, 1972; DALRRD, 1983). Additionally, any other foodstuff containing honey and/or bee products (royal jelly, propolis, beeswax) should comply with the Cosmetics and Foodstuffs, Act, 1972 (Act No 54 of 1972) as prescribed by the DoH (DoH, 1972). If honey or mixtures of bee products are imported, they should comply with the conditions of the permit issued by the Agricultural Pests Act, 1983 (Act No 36 of 1983) (DALRRD, 1983).

Tariffs are important for South Africa as a net importer of honey are barriers to international trade between countries which take the form of taxes levied by a government on imports, or occasionally on exports, for the purpose of protecting local industries or for generating revenue. South Africa generally has high import tariffs that make imported goods more expensive than domestically produced products (Ferreiria & van Wyk, 2017). The honeybee industry contributes R30 – 35 billion annually to South Africa's GDP, of which honey, beeswax and other hive products contribute R1 billion (Crewe, Masehela, Human, & Pirk, 2021). Similarly, exchange rate fluctuations are also implicated as a barrier in the trade relationship between countries.

Currently most honey is imported from China at a cost of $1,141 (about R15 900) a ton, versus double the price for Zambian honey ($2,375 or about R33 100) and close to $3,000 (about R41 800) per ton for Polish and Romanian honey. The exchange rate can have positive and negative repercussions for the local industries. The South African honey industry is negatively influenced by the bulk import of honey from China. Additionally, this honey is being portrayed as a high-quality product, but sold at a cheaper price. From the local South African perspective, a stronger rand is usually not beneficial to exporters as it leads to their export products being more expensive, but it is beneficial for importers as their product become cheaper (Crewe, Masehela, Human, & Pirk, 2021). The exchange rate can thus be viewed as a limiting factor which has a significant impact on profitability and consequently, on the production of honey (Ferreiria & van Wyk, 2017).

5.5.12 Covid-19 pandemic

In South Africa, the Covid-19 pandemic affected the honey industry. Movement of bees, beehive maintenance, as well as pollination services, were affected during the national lockdown (Level 5). Movement could only be done under strict conditions and had to be limited as far as possible (Crewe, Masehela, Human, & Pirk, 2020). Covid-19 affected the quality of honey and honey related products at a time when food scarcity was becoming a conservation concern. Beekeeping is a widespread and global activity, whereby communities depend on bees for their livelihoods and well-being. Together with wild pollinators, honeybees play a major role in maintaining biodiversity, ensuring the survival and reproduction of many plants, supporting forest regeneration, promoting sustainability and adaptation to climate change, and improving the quantity and quality of agricultural productions (Hendricks, 2021). Hives and bees could not be treated for diseases that also contributed to production, no treatment are employed in African honey bees. Providers of live bees such as Italy, New Zealand, Chili, California and Hawaii were affected because shipments were cancelled. Honey production and trade in China, who is a leader in honey production and royal jelly, were extremely affected. The United States of America pollination services for almonds were also affected (Crewe, Masehela, Human, & Pirk, 2020).

5.6 Conclusion and Recommendations

Multidisciplinary collaboration and enhanced dialogue are keys to develop consensus for change and joint vision towards sustainable food systems. Food safety standards are built on the following three pillars, namely: government, consumers, and industry and trade. It should also be taken into consideration that the food and agriculture sector consist of a complex web of activities, outcomes, and drivers.

Governments at all levels face increasing difficulty in their efforts to achieve sustainable food systems. This is primarily explained by the fragmented nature of the enforcers of the regulations. In South Africa the regulation of food laws is for the most part overseen by the ministries/departments DALRRD, DoH and DTIC. These ministries have a collective responsibility of ensuring food safety at national, provincial and municipal levels, which creates tremendous challenges, particularly when a break occurs in the food control chain of command. As the food safety and supply environments are rapidly changing across the world, South Africa’s multiple regulatory authorities have not been able to respond strategically and re-adapt in terms of integration and coordination. Furthermore, the presence of these multiple competent authorities and agencies involved in food control are rendered incompetent by fragmented legislation, organizational structure and functions. Governments play a key role in establishing policies and providing legislation that lays down minimum food safety or marketing standards that food businesses must meet. In addition, the government must ensure that food businesses comply with these requirements by means of training, inspection and enforcement. Cross sector collaboration and enabling regulatory frameworks, as well as technology innovation, all support a competent national food control system.

For this reason, collaboration between different public entities is necessary to ensure a streamlined food safety control system. Effective collaborative governance enables a better and shared understanding of complex problems involving many stakeholders and allows these stakeholders to work together and reach solutions. This leads to more innovation, efficient processes, increased success, and improved communication. This collaborative process can be long-lasting short-term, formal or ad hoc. The multi-stakeholder's collaboration mechanisms should be promoted to acknowledge the important roles of different food systems stakeholders (from production to consumption) in policy planning, implementation, and evaluation in South Africa.

In addressing the trends in the honey market, the revision of regulatory standardisation and methodology needs to be exploited and used. Regulatory standardisation of methodology and standards are important to ensure authentication of honey. In addition, it is also beneficial to use harmonized standards and methodology, as they ease the application of legislation, reduce the risk of conflict with other world trade organisation provisions, assist alignment with the Codex Alimentarius Standard for honey, and minimize TBT. However, there is a downside to regulatory standardisation that includes the fact that certain types of products and related information may be unique to specific countries or groups of countries and require special provisions; creation of potential conflicts with existing broader-based legislative requirements; cause confusion because consumers may be accustomed to receiving information in a particular format; in addition, changes even contravene local laws regarding the provision of information to consumers and cause language issues.

In our legislation honey is currently defined as a plant product on the local market and is made by honeybees and stingless bees. Hence, the APS Act is used as a monitoring tool. In the international honey market, honey is classified as an animal product and its quality is monitored as such. In addition, some markets are stricter when defining honey as honey that is only made from *Apis* species which results in trade barriers. Removing technological barriers to international trade can increase trade and improve a country's ability to export to the international market.

Botanical and geographical origin can be used as marketing tool for honey trading in both local and international markets. Origin is associated with higher quality or certain health benefits e.g., Manuka honey, as well as organic honey. Certain bee species are also associated with higher quality or certain health benefits, such as stingless bees, which are not native to South Africa. The domestic voluntary standard applied by the South African Organic Sector Organization (SAOSO) is currently not recognised by international certification bodies, and government has not adopted it as a mandatory organic standard in South Africa (Hendricks, 2021). Consequently, in addressing the increased demand for organic products, a specific national policy or regulation regulating the organic food sector is necessary. Currently, the production, processing and handling as well as labelling of organic products is required to comply with the general food regulations stipulated by the Food Control Division of the Ministry of Health and DALRRD. Hence, all organic foods imported and domestic are subject to the regulation relating to the labelling and advertising of foodstuffs. Organic products should also comply with the various DALRRD regulations relating to their quality, safety, grading, packing and marking as set out under the APS Act. Imported honey should be irradiated as part of disease control, which is, however, not the case at present because imported organic honey is not irradiated.

Authentication or adulteration detection methods are becoming issues of legal and commercial interest. The shortage of data on South African honey is affecting the monitoring of honey quality. In addressing this constraint, a comprehensive database of the quality of South African honey should be created. In doing so a substantial number of analyses of honey from all over South Africa for several years are necessary to determine the degree of variability in physical and chemical parameters due to seasonal, climatic and environmental condition. Pollen and various other analyses/tests should also be carried out to develop the database. Moreover, data of all types of honey e.g., botanical and geographical origin, nectar or honeydew honey, honey produced by different bee species, should be generated and stored in a database to enable the regulators to authenticate honey more rapidly and easily to stay ahead of the economically motivated adulteration processes. To ensure that South Africa's other related bee products are of appropriate quality, the compilation of standards for different bee products e.g., beeswax, propolis, royal jelly and pollen should also be investigated. The quality of stingless bee honey needs to be further investigated and implemented. This is a long-term project which is multi-disciplinary in nature and needs collaboration between multiply stakeholders.

In conclusion to assure an effective collaboration process, the multi-stakeholder's collaboration mechanisms should be promoted on both the national and the international level. Different types of institutions, regulations, subsidies, and laws influence food systems positively or negatively. To support this country having greater access to the international honey market, South Africa has to implement policies, legislation, and compliance measures regarding food quality control, the monitoring of sanitary and phytosanitary aspects along with the pesticides residues that address the trends in the national and international honey markets. As authentication of honey becomes more important globally, the South African honey legislation needs to be revised to address the ever-increasing risk of honey adulteration. To verify that bee products are of appropriate quality on the South African market, the regulatory framework and enforcement should be revised regularly. In South Africa, honey legislation is 20 years old and was only revised in 2000, in comparison to the Codex Alimentarius Standard for honey which was already revised three times in 1981,2001 and 2019 respectively.

5.7 Credit Statements

Tersia de Beer: Conceptualization, Methodology, Software, Former analysis, Resources, Investigation, Writing – Original draft.

Beulah Pretorius: Supervision, Conceptualization, Writing – Review and Editing.

Hettie Schönfeldt: Project administration, Supervision, Conceptualization, Visualization, Funding acquisition, Review, Structuring and Editing.

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5.9 Conflict of interest

The authors declare no conflict of interest.

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CHAPTER 6: SUMMARY, CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

Highlights:

- *Honey is a high-value, globally consumed, food product featuring a high market price strictly related to its origin.*
- *Honey characterization and authenticity is a complex issue which are influenced by production, trade, and consumer demand.*
- *The high demand for consumption and decreases in production of the honey increase the risk of honey to be falsified.*
- *Monitoring and evaluation of quality of honey on the South African market were evaluated using the Agricultural Standards Act as benchmark.*
- *The honey trade is faced with the challenge of honey authentication and traceability with regards to its botanical and geographical origin as well as adulterants.*
- *Physico-chemical parameters directly reflect honey quality and can be used to verify quality and detecting falsification of honey.*
- *South Africa's food control and safety system is a multi-agency system with three food control authorities namely, The National Department of Health, The Department of Agriculture, Land Reform and Rural Development and Department of Trade, Industry and Competition.*
- *The fragmented and silo attitudes of the multi-agency system challenges effective monitoring, control and implementation.*
- *A lack of cohesion decreases effective detection.*
- *Revision of legations are required to stay in line with current authenticity and adulteration issues.*
- *Research in honey is important to ensure food products of high quality and protection of consumer.*

Honey is the most important product from an apiary and is consumed globally, making it economically valuable (de Almeida-Muradian, et al., 2020). This study indicated that the production, trade, and sale of honey are a globalised business, which is driven by health-conscious consumers. Many consumers perceive honey as healthy, safe, and environmentally friendly (Pippinato, Blanc, Mancuso, & Brun, 2020). From a legislative point of view, the honey trade is currently regulated by a limited number of international standards based on the physico-chemical parameters set out in the Codex Alimentarius. The physico-chemical parameters prescribed are moisture (%), sum of fructose and glucose (%), sucrose (%), water insoluble (%), electrical conductivity mS.cm^{-1}, free acid (meq/kg), diastase activity (DN) (Schade scale), hydroxy-methyl-furfural (HMF) (mg/kg) and stable isotope ratio ^{13}C (‰) (Codex Alimentarius, 2001; Fakhlaei, et al., 2020; Tsagkaris, et al., 2021). At present, the regulatory and technical documentation globally governing the safety and quality of honey is not harmonised, which results in the adoption of a wide variability in the physico-chemical parameters to define honey and its quality standards. This makes the international honey trade susceptible to factors that are not strictly linked to quality aspects but are motivated by external factors that are political and economical (Thrasyvoulou, et al., 2018; Tsagkaris, et al., 2021).

The quality of honey is determined by various factors including bee species and the botanical- and geographical production environment (Tsagkaris, et al., 2021). Bees forage on different plant species from both natural and agricultural ecosystems. The *Apis mellifera scutellata* (*A.m. scutellata*) and *Apis mellifera capenis* (*A.m. capensis*) are responsible for honey production in South Africa. *A.m. scutellata* occurs naturally in the summer rainfall regions, whereas *A.m. capensis* is present in the western and southern Cape regions (Fynbos biome) (Eimanifar, Brooks, Bustamante, & Ellis, 2018). In addition, the *Apis* and *Meliponini* (stingless bees) bees show different preferences when foraging plant products, hence the compositional profile will differ (de Almeida-Muradian, et al., 2020).

6.1 Summary of main findings of this study

The vast number of honey types that are commercially available on the market are linked to market price and product quality. In addition, honey quality is related to origin and honey production. This makes honey a major target of fraud due to its price, high demand, and globalised market. These practices enhance the importance of food authenticity and traceability (Pippinato, Blanc, Mancuso, & Brun, 2020). However, determining the quality of honey is a complex task. Currently the main authenticity issues that are under investigation are botanical origin (44%), adulteration (21%), geographical origin (14%) and nutritive value (13%). Authenticity of honey has two aspects, verifying the botanical and geographical origin, as well as honey production (Tsagkaris, et al., 2021). In figure 6.1 the different authenticity issues are indicated in the honey value chain.

Various analytical tools were used to determine honey authenticity. The techniques mostly used for identification of the botanical origin included modern techniques and classical methods e.g., chromatographic methods combined with various detectors and all the physico-chemical parameters respectively. The physico-chemical parameters free acid, colour, viscosity and electrical conductivity were mostly used as identification markers, together with sensory analysis, and melissopalynological. Botanical, geographical and entomological origin was confirmed by spectroscopic techniques. Geographical origin was mainly determined by elemental profiles and to a lesser extent by melissopalynological, sensory analysis and isotopic techniques in combination with chromatographic techniques. Different molecular techniques were used to determine botanical and entomology sources. Adulteration was confirmed by vibrational and fluorescence spectrometry, isotopic techniques especially stable carbon isotope ratio, to determine addition of sugars. Other techniques include microscopic image identification, microbiological purity of honey, chromatographic techniques, electrical and rheological properties. Counterfeit honey was identified by various analysis namely sensory, physico-chemical, antioxidant activity and the presence of flavonoids as well as nuclear magnetic resonance spectroscopic analysis. Chromatographic, physico-chemical analysis, vibrational and fluorescence spectrometry and elemental analysis were used as analytical tools to determine nutritive value (Chin & Sowndhararajan, 2020; Tsagkaris, et al., 2021; Żak & Wilczyńska, 2023; Żak & Wilczyńska, 2023).

Countries have different regulations and legislation as part of their law enforcement efforts to monitor the quality of honey. This leads to different quality levels, different consumer expectations and different legal opinions regarding the labelling and definition of honey. In the European Union, the legal requirements are very stringent and while the Codex Alimentarius Standard for honey is acknowledged worldwide, the individual national regulations and controls throughout the world are quite different and not harmonised (Codex Alimentarius, 2001; European Commission, 2002). South Africa has a national standard, namely the APS Act, 1990 (No 119 of 1990) (DALRRD, 2000). In the APS Act, more physico-chemical parameters are prescribed to be tested than in the Codex Alimentarius and European Standards (EU). The APS Act has 17 physico-chemical parameters compared to the Codex Alimentarius 10 and European Standards (EU) eight (8) (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002).

In Chapter three the quality of honey on the South African market was evaluated using the APS Act, as assessment tool (DALRRD, 2000). The aim of the study was to evaluate and compare the quality of locally produced and imported honey available on the South African market from 1998 to 2017, using various physico-chemical parameters.

The honey was divided into regions (provinces) in South Africa and imported honey. The honey was further divided into forage types, namely, agricultural crops, indigenous forage, vegetation units and mixed flora. The study indicated a compliance of >80% for all honey types on the South African market. There was a significant difference between the quality of local and imported honey with the physico-chemical parameters of total acid, sucrose, and ash content as the parameters that distinguished most clearly between the groups. The imported honey physico-chemical parameter total acid was higher than the local honey, whilst the sucrose and ash content were lower. See Table 3.2. Honey from the agriculture crops differed significantly from the other forage types. The following physico-chemical parameters namely Lund, hydroxy-methyl-furfural (HMF) and ash content were identified as the physico-chemical parameters which usually distinguished between the forage types. The compliance which ranged from 80% to 94% with a mean of 90% and 92% for the local and imported honey samples, respectively, indicating that honey on the South African market is generally in accordance with national and international standards.

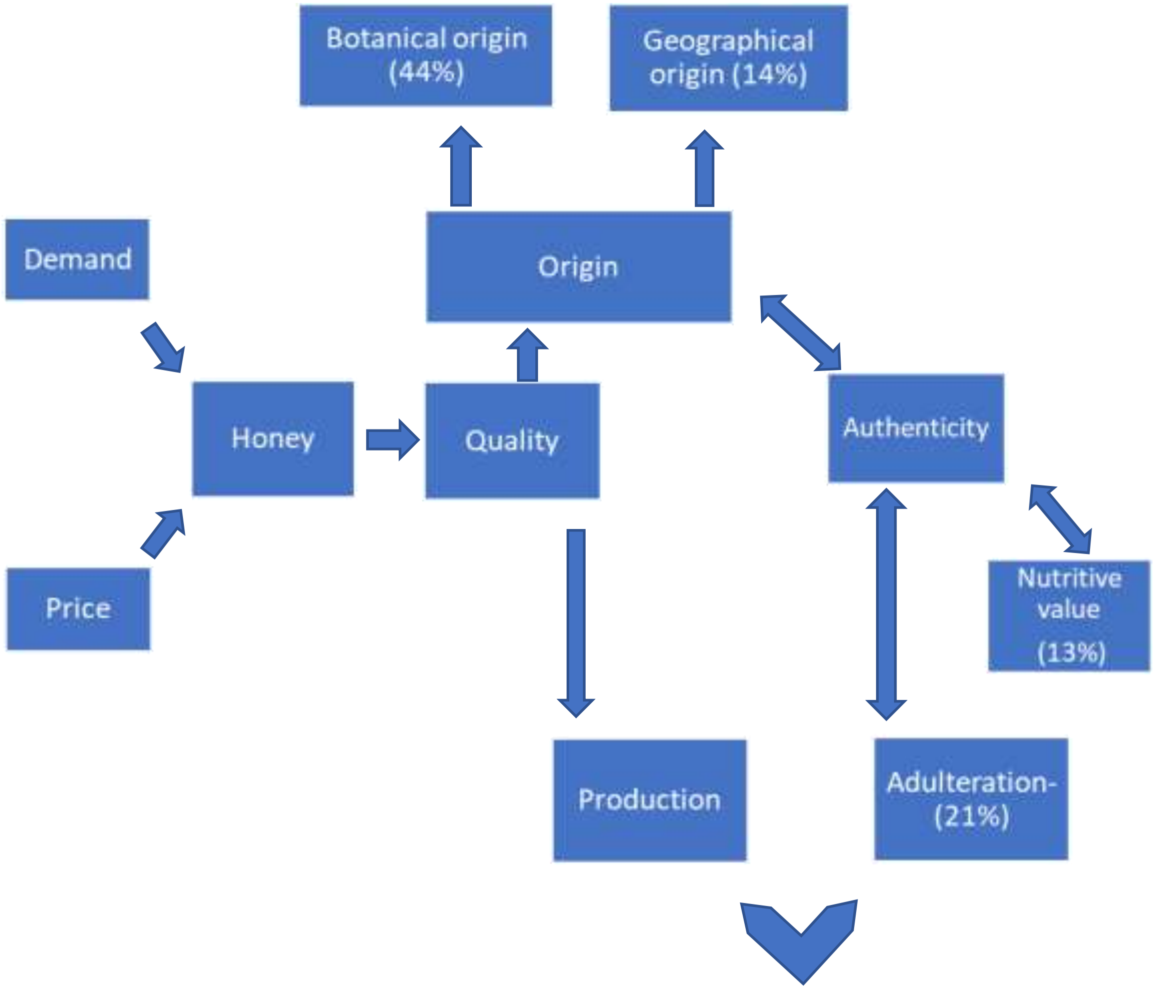

Figure 6.1: The different types of authenticity issues in the honey value chain, (Tsagkaris, et al., 2021)

Physico-chemical analysis is usually the first choice to determine the origin of honey. Honeys from different botanical and geographical origins have unique compositional profiles (Tsagkaris, et al., 2021). In Chapter four honey from different botanical (*Eucalyptus*, sunflower, aloe, sugar cane and sugar syrup) and geographical origins (Northwest, KwaZulu-Natal, Gauteng, Mpumalanga, and Limpopo), were included in the study for comparison purposes. In addition, an imported sample of Australian *Eucalyptus* honey was included for comparative purposes. The honey of different botanical origin is considered to be monofloral. Monofloral refers to honey derived from a nectar source of a single plant or plant species (DALRRD, 2000).

The lack of standards in the APS Act, regarding the physico-chemical characteristics of monofloral honey and the declaration of its geographical origin also influenced the quality of honey currently available on the South African market. Only certain countries, for example Germany, Greece, Serbia, and Turkey, have specific legislation for monofloral honey. Furthermore, Turkey has a set of physico-chemical characteristics for almost all the different types of monofloral honey produced in the country (Thrasyvoulou, et al., 2018).

In this study, selected physico-chemical parameter tests, and complementary tests such as the melissopalynological (pollen) and stable carbon isotype analyses were performed to authenticate honey to identify its origin (botanical and geographical) and potential adulteration (Figure 6.2). These selected physico-chemical parameters were chosen to determine the maturity, degree of deterioration, purity and adulterants. Physico-chemical analysis to evaluate the quality of honey is divided into the following categories namely 1) maturity, which includes analysis of reducing sugars, moisture, apparent sugars; 2) degree of deterioration which includes analysis of free acidity, diastase activity, HMF; 3) Purity which entails analysis of solids insoluble in water, minerals, ash and pollen and finally 4) adulterant-assessment, which includes the Legol-, Lund- and Fiehe Tests, and stable isotope analysis, which are the official methods for the detection of C_4-sugars (Salazar, Freitas, de Luz, & da Bersch, 2017; Fakhlaei, et al., 2020). The following tests are all prescribed in the APS Act, 1990: sugars (%) (Fructose, glucose, sucrose, maltose), Total acidity (free acid + lactone) (meq/kg), moisture (%), ash (%), Lund (cm^3), stable carbon analysis (‰). Optional tests not prescribed in the APS Act such as Refractive index, total soluble solids (°Brix), lactone/free acid ratio and the pollen test were also performed to assist in the authentication of the honey.

In addition to this study the specific compositional profiles were determined. The non-compliance of the physico-chemical parameters of the honeys were considered not to comply to the grading specification in the APS Act. With the melissopalynology analysis the botanical origin (*Eucalyptus*, sunflower, aloe, sugar cane and sugar solution) was confirmed, but the geographical origin could not be confirmed. One *Eucalyptus* honey sample originated from Australia. The stable carbon isotope analyses could not confirm botanical or geographical origin, nor sugar adulteration. The lack of standards in the APS Act, regarding physico-chemical characteristics of monofloral honey and the declaration of its geographical origin influence the quality of honey available on the South African market.

In Chapter five the South Africa food regulatory environment was reviewed with the focus on the determination of the quality of honey. In Figure 6.3 the overlapping of the three authorised food regulators in South Africa, namely the Department of Health (DoH), the Department of Agriculture, Land Reform and Rural Development (DALRRD), and the Department of Trade, Industry and Competition (DTIC), that are responsible for the quality and health aspects of honey, is illustrated.

Within South Africa the food regulatory environment is complex, as different authorities (DoH, DALRRD and DTIC) regulate different aspects of labelling, food safety, products standard(s) and facility design.

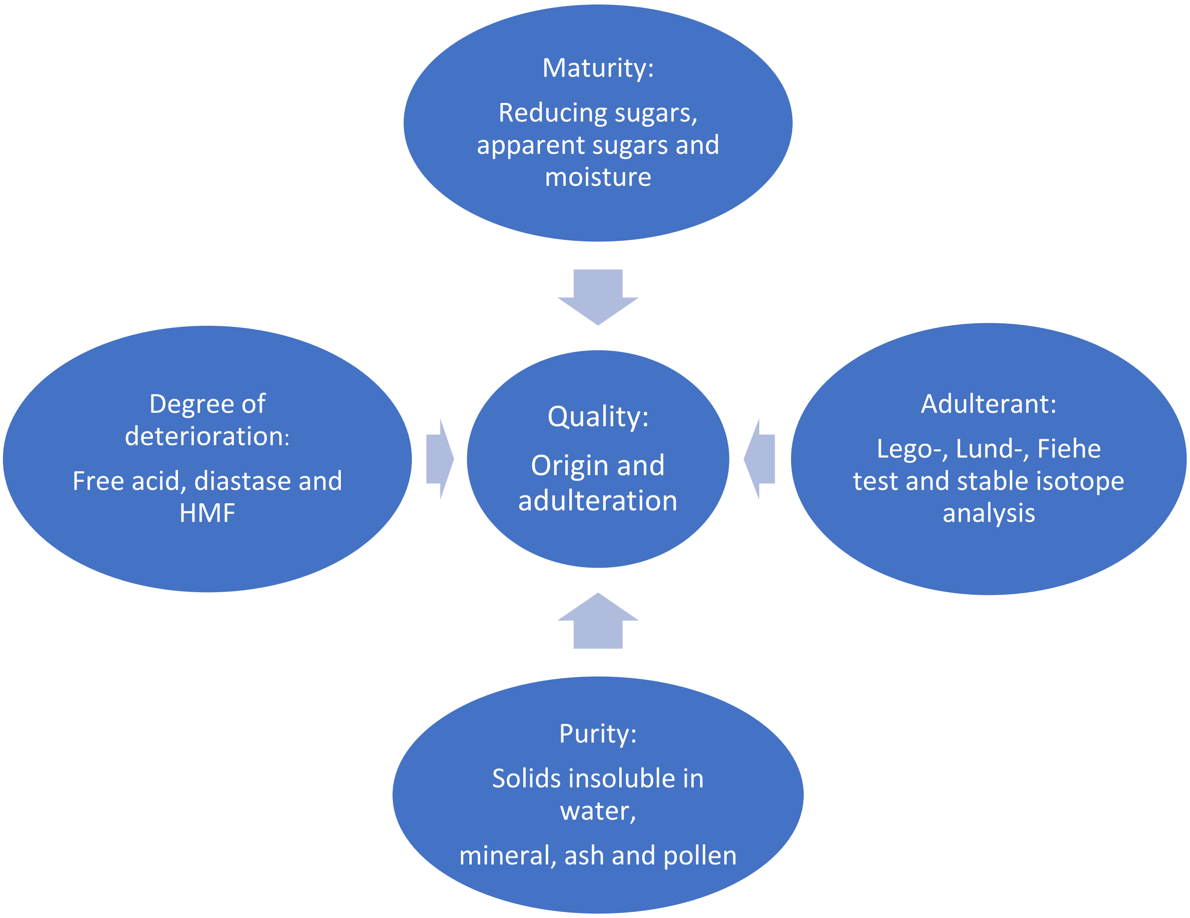

Figure 6.2: The physico-chemical analysis to determine maturity, degree of deterioration, purity and adulterants, (Salazar, Freitas, de Luz, & da Bersch, 2017)

In South Africa food legislation is mostly the responsibility of the health and agricultural sectors. These different departments in South Africa have different roles within the food regulatory system each with its own mandates and they are involved with different international institutions. National coordination is lacking between these departments because they concurrently have different specific mandates. This leads to financial and administrative constraints. Each department has its own representative for the different institutions that contribute to the financial burdens. The administrative challenges currently in the regulation of food safety regulation, are efficient regulatory activity, information sharing and effective risk assessment and management (Mukumba, 2011). Compared to the European Union, South Africa does not have a central body that regulates food safety regulations.

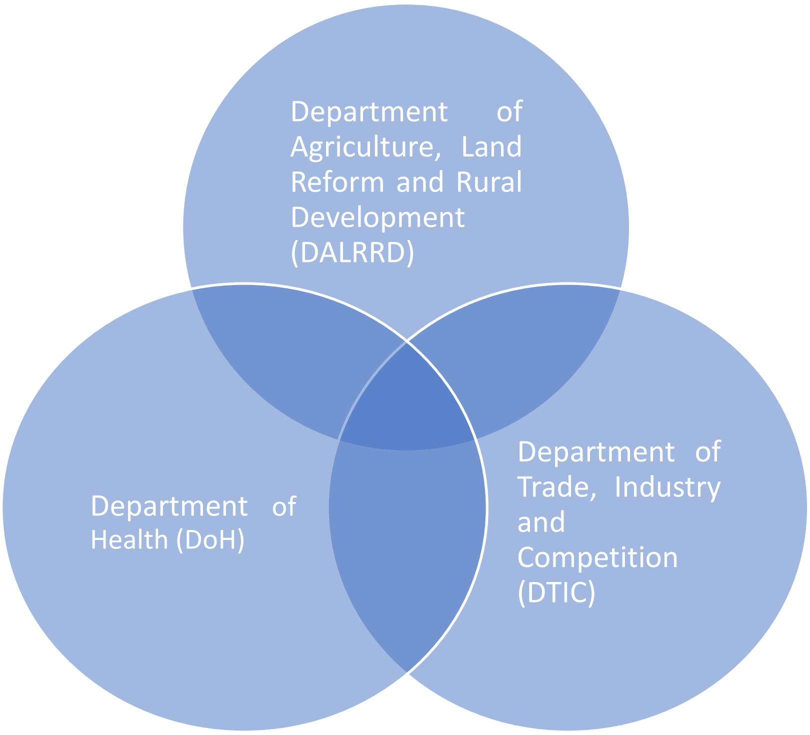

Figure 6.3: The three authorised food regulators in South Africa, namely the Department of Health, the Department of Agriculture, Land Reform and Rural Development, and the Department of Trade, Industry and Competition, (DoH, DTI, & DAFF, 2013)

The DoH is responsible for the overall coordination, determination of norms and standards, international liaison and cooperation, and provision of support to the provinces and on the local authorities' level (Sekgala, 2018). Furthermore, it participates internationally in Codex, the World Health Organisation (WHO), the International Food Safety Authorities Network (Infosan), the European Union Rapid Alert System for Food and Feed, and other African regional initiatives including the Southern African Development Community and the African Union (Mukumba, 2011).

Department of Agriculture Land Reform and Rural Development (DALRRD) is responsible for the implementation of Sanitary and Phyto-Sanitary (SPS) measures in South Africa which are carried out through Veterinary Public Health; Plant Health; Food safety and Quality Assurance; and the Agricultural Products Inspection Services. Moreover, DALRRD is the national body responsible for the monitoring and enforcement of certain aspects of food safety e.g., the quality of honey. The National Regulator for Compulsory Specifications (NCRS) is responsible for food safety regarding canned meat products, as well as the administrating of the Trade Metrology Act of 1973 (Act No 77 of 1973) and the Trade Marks Act, 1993 (Act No 194 of 1993), both of which are concerned with food labelling and guided by the Code of Hygienic Practice for Meat in the Codex Standards (Sekgala, 2018; DALRRD, 2000).

DoH

* Local manufacture, import, transport and sale of foodstuffs

* Food safety of foodstuffs

* Nutritional labelling

* Health related cliams and warnings

DALRRD

* Various directorates involved in production, export and import of plants and animals and products thereof

* Legislation covers production, quality, composition, labelling, food safety, biosecurtiy measures, etc.

NRCS

* Canned meat and meat products

* Frozen fish and other marine products

* Canned fish and other marine products

* Smoked snoek

* Live abalone

* Legal Metrology Act (Act 77 of 1973)

* Trade Marks Act (Act 63 of 1963)

Figure 6.4: Food Control System in South Africa, (DoH, DTI, & DAFF, 2013)

Codex is much more of a technical institution for developing international food standards, South Africa actively participates by making inputs, as well as compiling technical standards that might affect market access. This is a voluntary application, and countries use it as a basis for their national legislation such as for honey (Codex Alimentarius, 2001). Countries that use the Codex Alimentarius Standard for honey to compile their national legislation and use their recommendation will follow the international honey standard. The APS Act and the EU Directive 2001/110/EC used both the Codex Alimentarius honey standard as a basis (DALRRD, 2000; Codex Alimentarius, 2001; European Commission, 2002).

There are a variety of differences between honey standards and legislation that regulates the honey trade in and between countries. This can be attributed to the diverse characteristics that have been adopted in the different countries. These factors all contribute to unfair competition, misleading consumers about honey quality and commercial barriers and obstacles in honey trading. The benefits of regulatory standardisation are to ease application thereof and minimize Technical Barriers of Trade (TBT) and reduce risk of conflict with the provisions of other World Trade Organisations. In order to handle these challenges, the adoption of minimum requirements should be mandatory for all countries that produce, import and export honey.

6.2 Main conclusion/s of this study

The vast number of laws which need to be considered before honey can be sold on the local market, such as compliance with the Foodstuffs, Cosmetics and Disinfectants Act 1972 (Ac No 54 of 1972) and the Agricultural Pests Act, 1983 (Act No 36 of 1983) are confusing (DoH, 1972; DALRRD, 1983). Hence, the regulatory framework is often blamed for not being able to detect fraudulent practices or products on the market. The existing/available monitoring protocols have not been designed to detect new or unconventional adulterants. Consequently, many adulteration and food fraud incidents are not detected, which increases the actual prevalence of adulterated products. Therefore, the real number of adulterated products is not known (Koncz, et al., 2017). Currently, various methods exist to adulterate honey, consequently making it difficult to authenticate honey by using only one protocol /or guideline (Tsagkaris, et al., 2021).

As the results of the study indicate, multiple analyses which complement each other to characterise honey of different botanical and geographical origins and determine adulteration, failed to confirm origin and adulteration. The official method of stable carbon isotope ratio analysis (SCIRA), applied as described in Chapter four for determining sugar adulteration, failed. Non-compliance with the APS Act, does not mean that the analysed honey product is adulterated, nor does it mean it is in violation of the other two acts. Honey should be safe for human consumption according to the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No 54 of 1972) (DoH, 1972; DALRRD, 2000).

For honey to be safe, it should be free of contaminants and additives. Bees fall under the plant regulatory services and are, therefore, regulated in terms of the Agricultural Pests Act 1983, in addition the quality of evaluation is regulated in terms of the APS Act. The lack of data on honey origin and adulterations contributed to the fact that the current legislation cannot regulate the complexity of honey variations, hence in certain cases the parameters of authentic and unprocessed honey fail to comply with the composition criteria. Consequently, honey can meet the quality requirement, but as concluded in the study, this still does not imply that the honey is natural and/or authentic.

Food safety and quality should be a priority in all honey and mixtures of bee products. Adulteration of honey, especially with sugars, is a major problem. A honey adulteration case was exposed in 2018, in Kwa-Zulu Natal, South Africa. One of the protocols used to authenticate honey, is to determine the sugar profile. The honey samples where send to an external laboratory in Germany to determine possible adulteration of the honey with added sugars. The official method, called SCIRA was used for verification of adulteration. The principle of the official method for the detection of honey adulteration, is based on the stable carbon isotope ratio, $^{13}C/^{12}C$ of both sugars and inner protein content also indicates addition of C_4 sugars. Adulteration with sugars was confirmed by the isotopic results of the German laboratory. The $\delta^{13}C$ value of honey changes with addition of sugar causing a difference to occur between $\delta^{13}C$ values in honey and protein (Eshete, 2019a).

In South Africa the physico-chemical parameters could not confirm adulteration of the honey. Due to the lack of data on the compositional profile of different honeys (monofloral or polyfloral), contributed to the outcome of the results. The validity of the results received from the external laboratory in Germany is in doubt. The following reasons apply:

- A data library for honey from the Northern hemisphere (overseas) was used.

- The botanical and geographical origins differed between the honey tested and the information. The information we received relating to the data used for the evaluation of our sample of data used to evaluate it.

- Different bee species have different forage preferences. In South Africa we have the *A. m. scutellata* and *A. m. capenis* which also differ in geographical origin. Forage also differs because bees forage on the plants that are available in the vicinity of the hive.

- The determination of the carbon isotope ratio ($\delta^{13}C$/12C) and the degree of C_4 sugar adulteration (%) has been accepted as the means to detect C_4 adulteration. However, when honey has $\delta^{13}C$ values between -23.5% and -21.5% it falls within a grey area, as the carbon isotope ratios obtained for these samples suggest that these products may be adulterated, while those with a $\delta^{13}C$ value of ≥ -21.5% may be deemed to be adulterated (Eshete, 2019a).

- Legislative interpretation used in South Africa and the external laboratory differed. In South Africa the regulations for isotope analysis have been incorrectly specified and should be re-written. In the APS Act, the regulation states “the difference between stable isotope ratio ^{13}C (%) of the honey and the stable isotope ratio of its protein shall not be less than 0.0” (DALRRD, 2000). The protein acts as an internal control, given that its $\delta^{13}C$ value is unaffected by adulteration. In contrast, the $\delta^{13}C$ value of honey changes with addition of sugar causing a difference between the $\delta^{13}C$ values of honey and protein. A difference >1% in $\delta^{13}C$ values indicates that the protein and the bulk honey have different origins resulting in such a honey being classified as adulterated. In addition, honey with C_4 sugars <-7% can also be classified as adulterated.

- The distinct characteristics of honey types are influenced by the effect of environmental (floral and geographical origin) and processing (e.g., harvesting and storage) variables during production (Fakhlaei, et al., 2020).

In South Africa the quality of honey is monitored by the DALRRD by using the APS Act, a national standard, as assessment tool. All producers, packers and importers of honey and mixtures of bee products are urged to comply with the regulations relating to the grading, packing, and marking of honey or mixtures of bee products in the APS Act. However, the process of authenticating is very complex due to the great variation in the compositional profiles of honey. In this national standard various physico-chemical parameters are prescribed to monitor the quality of honey. When one of the physico-chemical parameters is in non-compliance of these required physico-chemical parameters it is deemed as not complying with the standards of grades of honey. In the international standard, namely the Codex Alimentarius Standards for honey on the other hand, if a non-compliance of one of these physico-chemical parameters occurs the honey is considered to be adulterated.

6.3 Limitations of this study

The following limitations of this study must be considered:

- The findings of this study are only relevant to the study of the honey on the South African market, which includes imported, local, and blended honeys that were tested over a prior of 17 years by the Department of Agriculture, Land Reform and Rural Development (Chapter three).

- Mislabeling of botanical origin is considered an indirect form of adulteration. Samples were grouped according to their floral origins (agricultural crops, forestry, indigenous genera, vegetation units and mixed flora) as were indicated on the label of the local samples. (Chapter three (3). Most of the honeys on the market are polyfloral honeys as the bees forage on the flowers in their surroundings. “Bees forage within two – five kilometers of their hives, while they can travel as much as 14.4 kilometers from the hive”.

- Another way of indirect adulteration is the mislabeling of geographical origin. Samples were grouped together according to the address on the label of the containers. The honey samples were from different regions in South Africa: Kwa-Zulu Natal (NTL), Northern Cape (NC), Eastern Cape (EC), Gauteng (GP), Free State (FS), Northwest (NW), Limpopo (LIM), Mpumalanga (MP), unknown origin (UNK) and Western Cape (WC). In addition, the imported honeys originated from China, Argentina, Romania, India, Zambia, Egypt, Kuwait, Lesotho, Singapore, Australia, New Zealand, Zimbabwe and from unknown origin.

- All regulatory frameworks have limitations. The standards used to determine the quality of the honey on the South African market are all based on the standards compiled for the Northern hemisphere, European origin, as well as on the honey produced from *A. mellifera* honeybees. This is an inherent predicament as this set of quality standards for South African honey has been established with honey from other countries. Honey of stingless bees differs regarding the viscosity, colour, taste, lower sugar content, and a higher moisture content (Razali, et al., 2018; Sahlan, et al., 2019).

- The various physicochemical parameters determining the quality of honey have their own potential failings that may limit the validity of the research findings. Various factors influence the different physicochemical parameters such as botanical- and geographical origin, environmental factors, beekeeping, and handling techniques (overheating and storage) that directly affect honey quality (Chin & Sowndhararajan, 2020). The physico-chemical parameters identified to be tested in accordance with the APS Act, are sugars (sucrose, fructose, glucose, and maltose), fructose/glucose ratio and reducing sugars, total acidity, moisture, HMF, ash, Lund and specific rotation. If results of any of the above-mentioned tests are non-compliant with the specifications of the South African honey regulations, this is considered as non-compliance with the grades of honey, while in comparison to the international standards non-compliance is considered as adulteration (DALRRD, 2000).

- Entomological sources. The EU Directive standard specify that honey has to be produced by *A. mellifera* bees differentiating it this way from the honey that is produced from other species *(Micrapis, Megapis, Meliponines)* (Thrasyvoulou, et al., 2018; European Commission, 2002). According to the Codex Alimentarius Standard for honey, honey is made by all honeybees (Codex Alimentarius, 2001). Both the EU Directive and Codex Alimentarius Standard for honey indicate honeydew honey is made from the excretions of the plant sucking insects Hemiptera (Codex Alimentarius, 2001; European Commission, 2002). While the APS Act states that honey is made by honeybees (Apis Genus) or stingless bees (Melliponinae order). In addition, it is not specific about which plant sucking insects produce honeydew (DALRRD, 2000).

- Depending on which standards are applied EU Directive or Codex Alimentarius the definition of honey might differ. As true honey is defined as honey made from only the *Apis* spp.

- False declaration of processing conditions, such as irradiation, is also a form of honey fraud. The indication of irradiation is a phytosanitary requirement and is often not indicated on the labels of the honey containers. In the case of blending local honey with imported honey, irradiation is also not declared on the label. All imported honey should be irradiated according to the DoH and DALRRD regulations. Irradiation of imported honey into South Africa is to prevent the spreading of the pathogen *Paenibacillus larvae* (DALRRD, 1983) Zambian honey is exempted from mandatory irradiation due to an agreement made in 2015 between the South African and Zambian governments after the Zambian honey complied with the set of phytosanitary requirements. Furthermore, because irradiation makes changes to organic honey, it is not irradiated (DoH, 1972; DALRRD, 1983). To date, only limited studies are available on the relationship between irradiation and honey composition. The effect of irradiation on the entire chemical composition of honey has also not been studied comprehensively. In a study done by Aly and co-workers, (2021) honey treated with a dose of 10.0kGy of gamma rays did not significantly affect the physico-chemical parameters, namely ash content, pH value, total soluble content, and sugars, however the moisture content decreased, while the colour, phenolic content, flavonoids and antioxidant activity increased. The HMF value was lower in contrast to the diastase activity (Aly, Maraei, Abd-Allah, & Safwat, 2021).

- The quality of honey is affected by variations in pre- and post-production processes of honey, as well as the varies adulteration modes. The processing methods used during honey production include, heating, freezing, radiation treatment, filtration and centrifugation, and are used to meet the consumer expectation of a uniform and liquid product. However, if these processes are not done according to set standards, then the quality will be affected. Sophisticated adulteration processes are used to limit the detection of adulterants. Adulterants added to honey will affect the chemical and or physical properties of honey. Adulterants such as sugars from C_3 plants (coconut, rice, and sugar beet syrups), adding pollen and adding water are some adulterants that are difficult to detect (Tsagkaris, et al., 2021).

With no and limited standards available, it is not possible to detect these added adulterants according to the APS Act. However, simple adulterations with commercially available sugars with unaltered composition can be easily detected, for example, sucrose (DALRRD, 2000). Adulteration entails the following: directly adding commercial syrup, cane and other sugars to honey, or indirectly feeding bees with a concentrated sucrose solution within the beehives. Less common is the harvesting of unripe or green honey, which can be forcibly dehydrated to comply with the set standard of moisture (Fakhlaei, et al., 2020). This a violation of the Codex Alimentarius Standard for honey (Codex Alimentarius, 2001). Ion-exchange technology to remove pollen, pesticides, antibiotics, and colour particles to mask the origin of adulterated honeys is also used.

- The stable carbon isotope analysis used to confirm adulteration of honey samples in South Africa has some limitations. It is not validated nor accredited, there is only one facility in South Africa that performs this analysis, and only the sugar content of the bulk honey samples could be determined and not the separate sugars, as well as the protein content. Hence adulteration could not be confirmed for the botanical or the geographical origin of the sugars (Chapter four).

- The sample size of the study was limited by research funding and capacity available to determine the authenticity of local honey. The choice of honey samples was based on the honey that is mostly consumed by consumers e.g., eucalypts, sunflower and to a lesser extent, aloe. Sugar cane and a sugar syrup were added as control samples (Chapter four).

- While farming is considered an essential service, bee-keeping activities could continue but under limited conditions. In South Africa bees are not considered “livestock”, DALRRD has, therefore, insisted that bee-keeping activities, especially movement of bees and hives, should be kept to a minimum. During the Covid-19 Pandemic, permits were issued to the South African beekeepers and bee removal operators to work outside the lockdown conditions. Small scale operations came to a halt. Because bees are also pollinators of agricultural crops delaying the movement of bees and hives had a big financial impact on beekeepers and the country’s imports and exports as the declining income affected households and caused shortage of forage. No provincial cross border movements were allowed until lockdown level 3 was announced, as well as the curfew from 20h00 pm to 5h00 am. (These restrictions influenced other activities as well, namely staff recruitment, staff safety and transport, equipment supplies, migratory beekeeping/pollination services, shipments of Queens/nuts, meetings, sales, and husbandry elderly beekeepers. Other types of impacts included negative attitudes of workers, vandalism and bee removals that were not done properly) (Crewe, Masehela, Human, & Pirk, 2021). In contrast, online tasks could continue.

6.4 Recommendation/s

The recommendations of this study are set out below. The quality of honey is determined by its taste, consistency, and aroma, which are also the most important parameters that affect the acceptability of the product by the consumer (Karabagias & Karabournioti, 2018; Tsagkaris, et al., 2021). In addition, honey must meet certain qualitative criteria to be considered as a high-quality product (Warui, et al., 2019). However, although these physico-chemical parameters are valid all over the world, they do not satisfy all the set standards of all countries.

- Given that farming (including beekeeping) is considered an essential activity, it was permitted to continue during lockdown, however under limited conditions. **It is suggested that policy makers should also consider farming-related research as essential, and regard bees as “livestock”**, so that special regulations can be developed for field and laboratory staff to allow continuation of their work while ensuring health and safety even during Pandemics (Crewe, Masehela, Human, & Pirk, 2021).

- Measures should be taken to ensure that the sufficiency, sustainability, safety, and integrity of foods are more closely linked than ever before. Hence, we should stay alert to prevent the illegal and possibly hazardous food products from entering the market because of shortages created by secondary factors, for example the COVID-19 Pandemic. In such times when sufficiency is critical, it is important to avoid preventable food recalls due to authenticity concerns. **The South African government should consider a more streamlined food regulatory environment to enforce regulations**. The complexity of the South African food regulatory environment should be simplified to ensure a better regulatory compilation and enforcement of set standards such as labelling, food safety, product standards and facility design. This will facilitate further collaboration and assist in identifying gaps in honey production and the regulatory framework. Only with joint efforts will there be better resource use and efficiency in convening research to bring about policy change.

- The increasing demand for natural foodstuffs and the lack of organic food standards are forcing DALRRD to compile standards for foods that are organically produced. **Priority must be given to finally reach agreement on the proposed organic standards and the implementation thereof, and allocation of an authority to oversee these organic standards**.

- New and better analytical methods are available nowadays, which are fast, reliable, simpler, accurate, non-destructive, more economical and can detect numerous adulterants. **The introduction of new norms, using these new methods is necessary to detect fraudulent products**. These methods can be applied to determine the authenticity of honey using targeted and non-targeted approaches. In a targeted approach, the compound of interest is known, whereas screening is used in the non-targeted approach. These methods are specific in determining the chemical fingerprints e.g., isotopic signature and biologically active components and for evaluating the biochemical constituents such as polyphenols and flavonoids of authentic and artificial honey. The application of harmonised and validated test methods to detect adulteration that are prescribed by international authorities should be incorporated into South African legislation. Continual research in screening techniques is necessary.

- **Sensory analysis of honey** describes the organoleptic profile and **may be used to assess the quality**, **as well as the botanical and geographical origin** of a honey sample. Furthermore, sensory evaluation, in addition to physico-chemical and melissopalynological analyses, is essential for determining uniflorality. Moreover, organoleptic characteristics are the only ones that consumers can identify and evaluate. Trained specialists are, however, required to evaluate honey based on their sensory experience. **To address the lack of skilled analysts, more modern techniques (instrumental) such as e-tong and e-nose should be applied more regularly**.

- To help prevent misrepresentation of the classification and identification of the geographical origin of honey it can be **classified according to the area the honey was harvested** in. **It is suggested that the following wording should be added to honey descriptors "blend"** for example "blend of South African honeys", "blend of South African honeys with honeys from European countries" or "blend of South African honey with honeys from non-European countries" or "blends of South African honey with honey from a specific country e.g., Chinese honey" or "blends of South African honey with honey from Southern Africa Development Community countries". In the case of honey products where bees were fed sugar solutions during a dry season or winter to ensure their survival, it should be indicated as "sugar-syrup or honey blend with sugar solution". This honey should be monitored on a regular basis.

- **Honey labelling** should state the country of origin of the largest portion and then continue listing the other **countries of origin in decreasing order** as depicted on the labels of other food stuffs.

- The **traceability** of honey is of the utmost importance for authentication of honey, as well as food safety. Hence improvement of traceability systems is vital in combating honey fraud. It can be **achieved by adding additional information to the labels**. This additional information can include the registration number of the beekeeper, the beekeeping association per province, or a possible website specifically for honey for the consumer to investigate the history of the beekeeper and complain if necessary. If honey is suitable to be called a baker's honey it should be described as such on the label. Surveys should be carried out on a regular basis with beekeepers to improve the traceability of honey.

- **Removal of the words "pure" and "natural"** honey as they confuse consumers. Honey is inherently natural. Instead, the grading can be used more efficiently. If the sensorial and colour analyses are added as part of the quality evaluation the grading of choice or industrial grading will be more effectively assessed and it will also give the consumer greater confidence in the product. The different **grades of honey and correct origin must be clearly defined** to prevent misrepresentation and unfair competition, such as when a cheap imported honey is marketed at the incorrect grade.

- The European Union honey regulation contains a definition for industrial honey, whereas, the Codex standards and the APS Act, have no such definition. In the APS Act, industrial honey is only mentioned as part of the grades. **It would be beneficial to add the definition of industrial honey in the APS Act.** In addition, **baker's honey**, which is used in confectionary, is also not mentioned and **should be defined as industrial honey**. This is confusing as no applicable standards for baker's honey are available.

- To ensure the use of harmonised standards and legislation internationally in the honey trade, **clarity on honey categorisation should be made clear**. On the **international market honey is classified as an animal product, while in South Africa it is classified as a plant product** (DALRRD, 2000; European Commission, 2002). This discrepancy in classification causes a technical trade barrier for export. If honey is exported to the EU, the rules for animal production and trade apply. In addition, a health certificate must accompany each batch of honey to ensure compliance with EU animal health requirements. This certificate must be issued by a competent veterinary authority and signed by an official veterinarian.

- The South African government together with all relevant stakeholders should embark on a long-term project to **determine honey standards for honeys produced in South Africa that will assist in the authentication**. It is essential for transparency and trust between all stakeholders to ensure the success of this project. This group of stakeholders includes research institutions, the government, non-governmental organisations, civil society organisations, health care workers, the food industry, and community representatives. **Dialogue should be directed to establish an action plan to improve the quality of honey** in this period of declined honeybee farming in South Africa.

- In South Africa food safety legislation is mainly the responsibility of the health and agricultural sectors. Furthermore, South Africa maintains a sophisticated and well functional food safety regulatory framework, however this decentralised approach has led to a complex institutional framework and lacks co-ordination. **The establishing of one food safety authority to monitor food safety would be beneficial for the honey industry**, as well as the consumer. A simpler regulatory authority with a single contract for stakeholders could increase the effective and efficient monitoring of the producers and honey products. Overlapping of legislation contributes to higher financial costs and a less effective detection of non-compliant products e.g., adulterants, food safety risks and identifying if an imported product is irradiated or not.

- The results of the online survey disseminated through the global COLOSS honeybee research association, showed that **enhanced support is needed for future research on the impact of pandemics** on bee research and its products. **Funding should be made available** by all stakeholders to ensure that in future pandemics, this can be addressed more efficiently, so as to limit their negative impact.

- **Regular revision and amendments to legislation and standards regarding honey authenticity** will lead to more effective application thereof. The last revision of South African honey regulations was in 2000. Consideration needs to be given to re-instate Government Notice No. R. 2336-19 October 1979. As addition to the current regulation namely R835-25 August 2000 in terms of the APS Act (DALRRD, 2000). In Government Notice No. R. 2336- 19 October 1979, standards such as values for "Brix, color, pollen test, taste, percentage of honey to be added for blending, standards for grading" are all mentioned. Unfortunately, they were removed during the last revision of honey regulation in 2000. In support of increased effectiveness in the application of regulatory enforcement of standards that govern honey production and quality control on the South African market, consideration should be given to re-introduce the standards that were removed in the previous revision of the honey regulation. This will also be beneficial to assist with compiling compositional profiles of monofloral honeys. This will also contribute to the compilation of national standards for monofloral honeys.

- The final recommendation of this study relates to the lack of a development modelling program/ database of honey quality. To improve the capability of authenticating honey, it is recommended that a **compositional database of authentic honeys and of substances which may be added to increase its volume or bulk, or which are used as bee feeding products, should be created**. This would require accumulating samples representative of domestic honey production. The selection and sampling of the honey would have to be performed under the supervision of competent authorities. It is recommended that all stakeholders and researchers should take part in the development of such a database and intensify their efforts for funding to develop such as database and enable them to upgrade and distribute the program more widely.

6.5 Conclusions

The demand for honey appears to be ever-increasing, therefore, the risk of honey fraud is also on the rise. Local honey regulations play an integral part in combatting honey fraud. These regulations should be clear and relevant and should be updated as new scientific advances are made. The lack of standards in the APS Act, regarding the physico-chemical characteristics of monofloral honey and the declaration of its geographical origin influences the authentication of honey. The use of multiple approaches, which complement each other would be more reliable than the present methods to characterise honey of different botanical and geographical origins. Finally, the regulation should provide definitive testing requirements, encompass recent scientific findings when setting these requirements and should take into consideration the current and relevant concerns of the bee industry. If it is not feasible to standardise a test for the botanical and geographical origin of honey, therefore, stricter requirements should be implemented regarding labelled origin claims and the documentation that is required to substantiate these claims. In view of the fact that these regulations were published over 20 years ago, this highlights the need for the regulations to be reviewed and updated.

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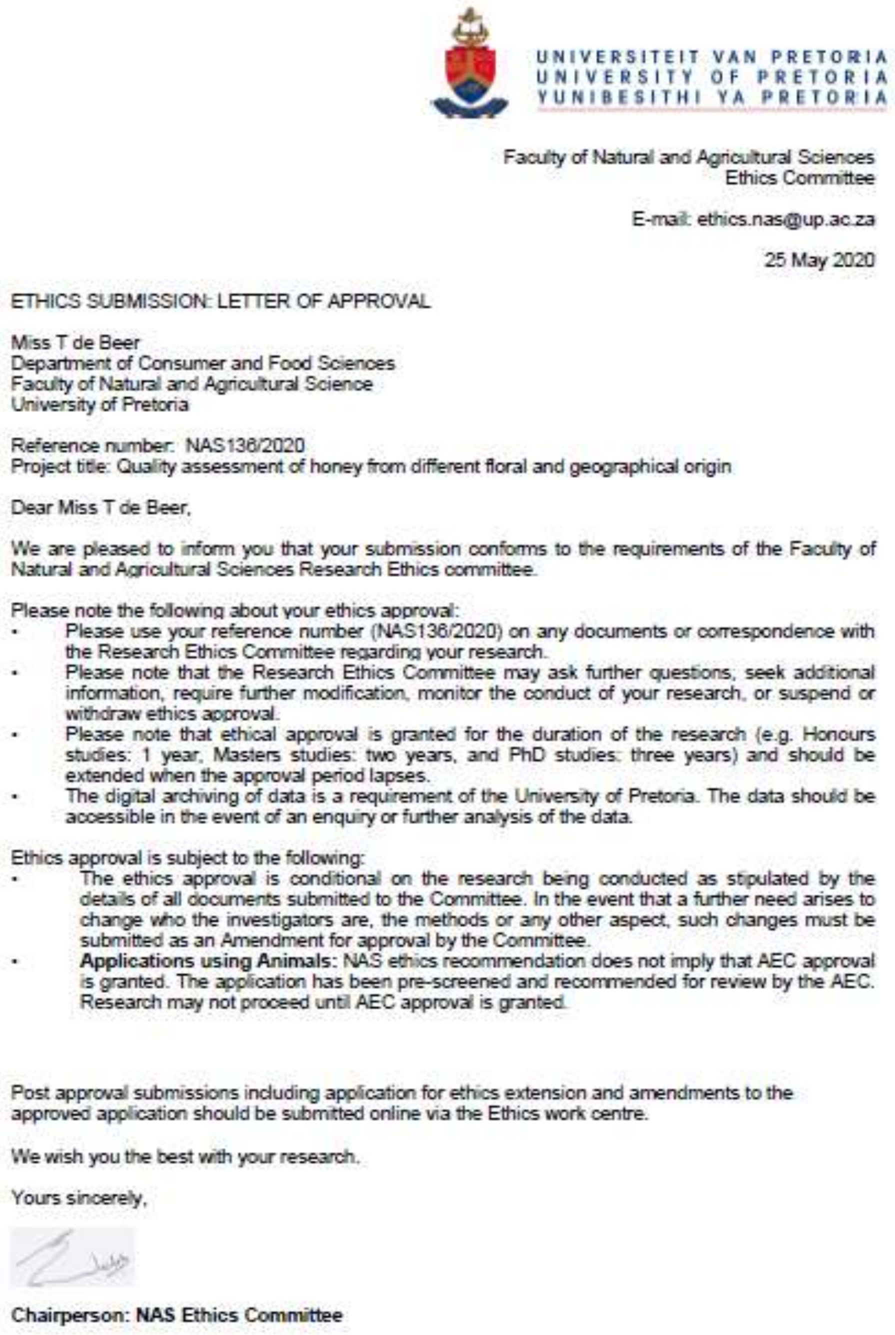
APPENDICES

Appendix 1: Ethics approval letter.

Ethical clearance was granted by the University of Pretoria ethical committee at the start of this project.



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Natural and Agricultural Sciences
Ethics Committee

E-mail: ethics.nas@up.ac.za

25 May 2020

ETHICS SUBMISSION: LETTER OF APPROVAL

Miss T de Beer
Department of Consumer and Food Sciences
Faculty of Natural and Agricultural Science
University of Pretoria

Reference number: NAS136/2020
Project title: Quality assessment of honey from different floral and geographical origin

Dear Miss T de Beer,

We are pleased to inform you that your submission conforms to the requirements of the Faculty of Natural and Agricultural Sciences Research Ethics committee.

Please note the following about your ethics approval:

- Please use your reference number (NAS136/2020) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
- Please note that ethical approval is granted for the duration of the research (e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
- The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
- **Applications using Animals:** NAS ethics recommendation does not imply that AEC approval is granted. The application has been pre-screened and recommended for review by the AEC. Research may not proceed until AEC approval is granted.

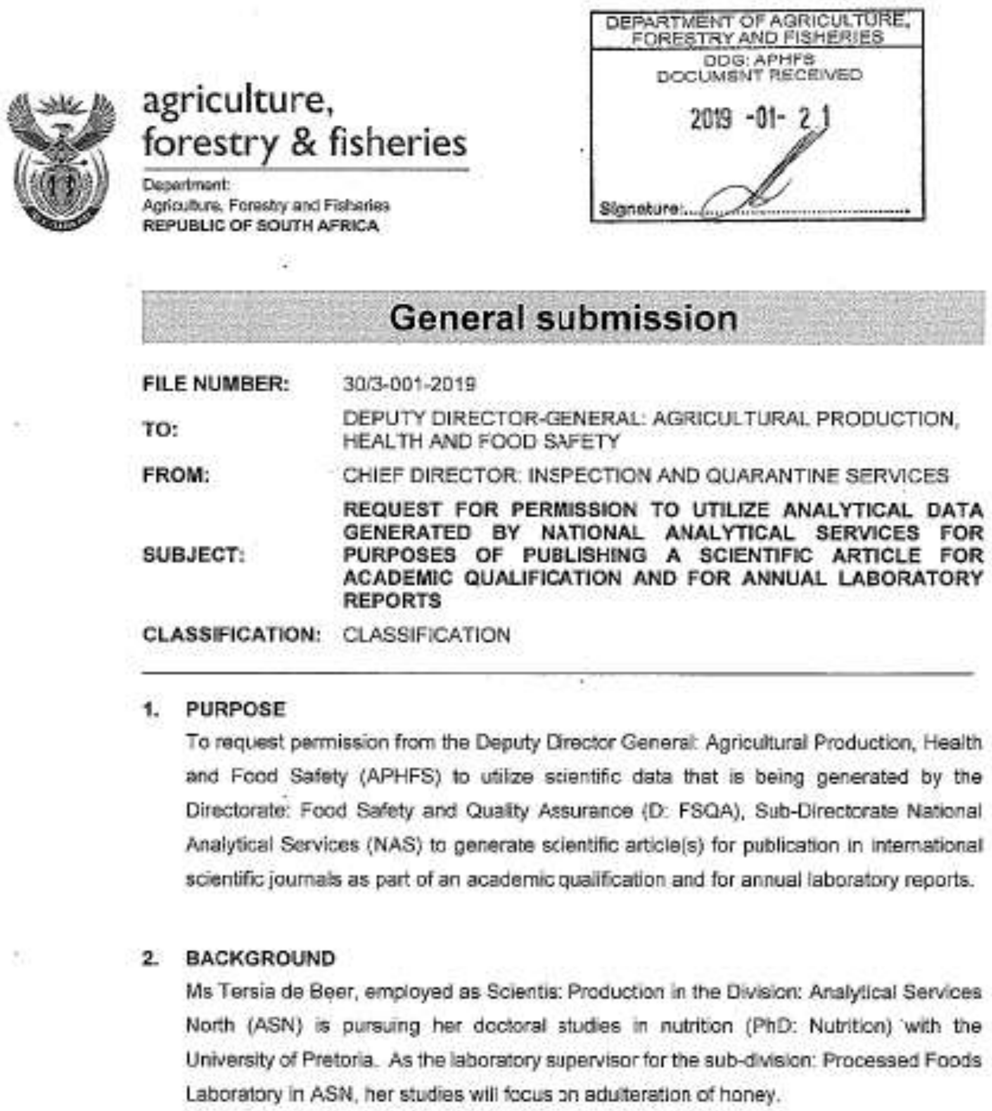
Post approval submissions including application for ethics extension and amendments to the approved application should be submitted online via the Ethics work centre.

We wish you the best with your research.

Yours sincerely,

Chairperson: NAS Ethics Committee

Appendix 2: Approval letter from the Department of Agriculture, Forestry and Fisheries to utilize the analytical data generated by the National Analytical Services for purposes of publishing.



agriculture,
forestry & fisheries
Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES
DDG: APHFS
DOCUMENT RECEIVED
2019 -01- 21
Signature:

General submission

FILE NUMBER: 30/3-001-2019

TO: DEPUTY DIRECTOR-GENERAL: AGRICULTURAL PRODUCTION, HEALTH AND FOOD SAFETY

FROM: CHIEF DIRECTOR: INSPECTION AND QUARANTINE SERVICES

SUBJECT: **REQUEST FOR PERMISSION TO UTILIZE ANALYTICAL DATA GENERATED BY NATIONAL ANALYTICAL SERVICES FOR PURPOSES OF PUBLISHING A SCIENTIFIC ARTICLE FOR ACADEMIC QUALIFICATION AND FOR ANNUAL LABORATORY REPORTS**

CLASSIFICATION: CLASSIFICATION

1. PURPOSE

To request permission from the Deputy Director General: Agricultural Production, Health and Food Safety (APHFS) to utilize scientific data that is being generated by the Directorate: Food Safety and Quality Assurance (D: FSQA), Sub-Directorate National Analytical Services (NAS) to generate scientific article(s) for publication in international scientific journals as part of an academic qualification and for annual laboratory reports.

2. BACKGROUND

Ms Tersia de Beer, employed as Scientis: Production in the Division: Analytical Services North (ASN) is pursuing her doctoral studies in nutrition (PhD: Nutrition) with the University of Pretoria. As the laboratory supervisor for the sub-division: Processed Foods Laboratory in ASN, her studies will focus on adulteration of honey.

Objectives of Ms de Beer's studies are the evaluation of (i) the quality of honey currently available on the South African market, (i) the quality of agriculture crops, forestry and indigenous forage honey types as part of authentication of honey types and (iii) analytical methods as part of the support to legislation surveillance and enforcements. It is laboratory test results data which will be used for this study and anonymity of producers/manufacturers, persons and departmental units will be kept. The analytical data used will be for profiling and characterisation of products and interpreted against existing regulations.

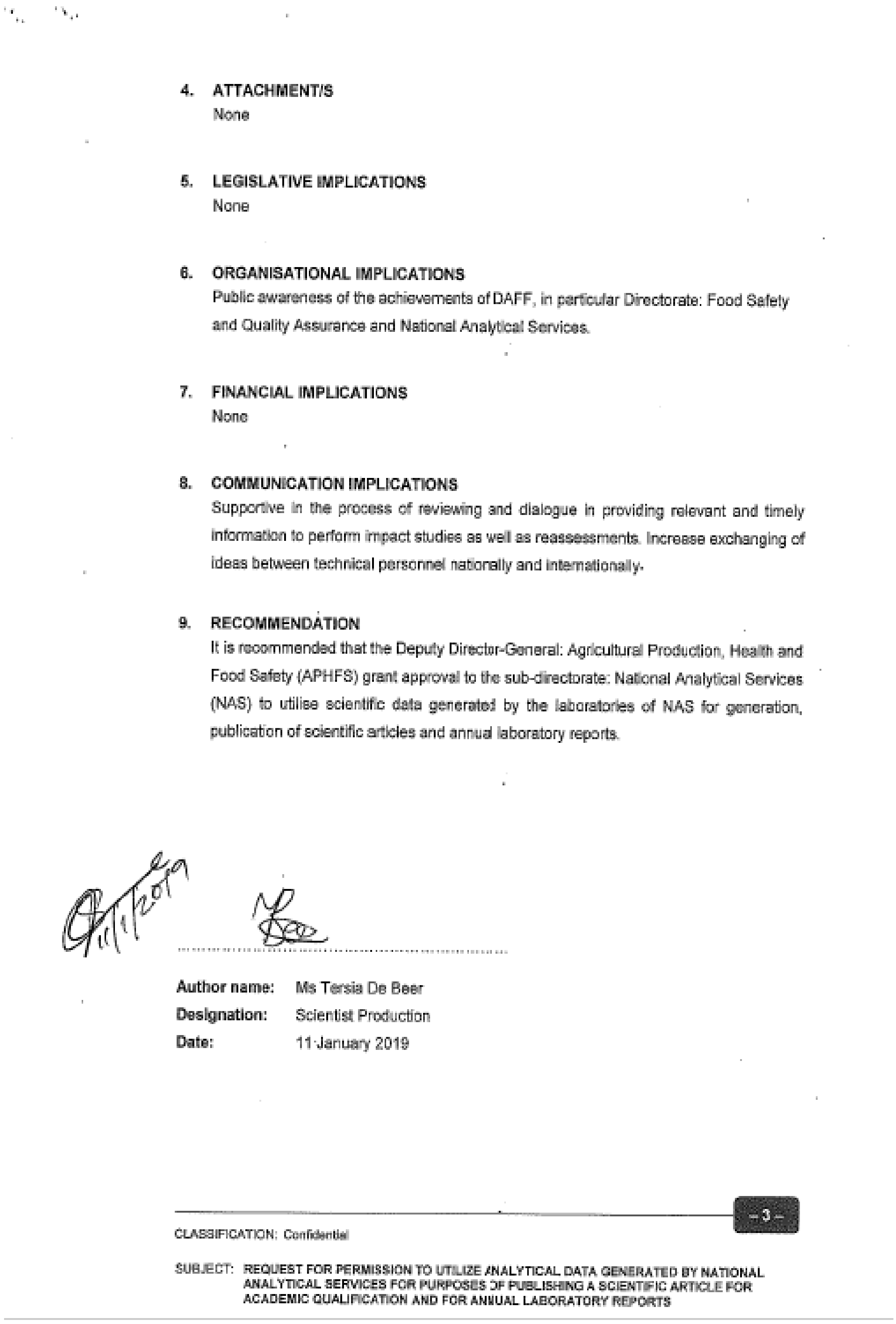
The Department of Agriculture, Forestry and Fisheries (DAFF), has the responsibility of implementing sanitary and phyto-sanitary measures as well as monitoring and applying regulations to ensure the quality of agricultural products such as processed foods and dairy products. The regulatory mandate is the Agricultural Product Standards Act 1990 (Act 119 of 1990). This is currently administrated by the Directorate: Food Safety and Quality Assurance (FSQA).

3. DELIBERATIONS

The role of governments is to adopt and monitor compliance with national food legislation, as part of protecting the public's health. The enforcement of these norms are based on the specific needs of the specific market in this case, South Africa, and are usually harmonised with international standards.

Testing capabilities are an important tool which enables governments to monitor compliance of products to regulations thus facilitates enforcement as well. Training of Ms de Beer, especially in addressing objective (iii) the evaluation of analytical methods supportive of the surveillance and enforcement of adulteration of honey – through the exploration of utilising the Fourier Transform Near Infrared (FT-NIR) and the Isotope Ratio Mass Spectrometric (IRMS) techniques. Working with an expert in IRMS at the University of Pretoria who is currently conducting tests to authenticate the quality of honey, the incumbent will be capacitated and be empowered to implement a similar method in ASN.

Furthermore, the ASN endeavours to publish annual reports reflecting the status of compliance for products tested by the laboratory. It is imperative to provide feedback as regulatory body. An annual report or publications in scientific journals are some of the avenues available to the Directorate: FSQA to communicate status of compliance towards ensuring the consistent quality monitoring of regulated products offered to consumers in South Africa. All publications, annual reports and scientific articles will remain the intellectual property of DAFF.



4. ATTACHMENT/S

None

5. LEGISLATIVE IMPLICATIONS

None

6. ORGANISATIONAL IMPLICATIONS

Public awareness of the achievements of DAFF, in particular Directorate: Food Safety and Quality Assurance and National Analytical Services.

7. FINANCIAL IMPLICATIONS

None

8. COMMUNICATION IMPLICATIONS

Supportive in the process of reviewing and dialogue in providing relevant and timely information to perform impact studies as well as reassessments. Increase exchanging of ideas between technical personnel nationally and internationally.

9. RECOMMENDATION

It is recommended that the Deputy Director-General: Agricultural Production, Health and Food Safety (APHFS) grant approval to the sub-directorate: National Analytical Services (NAS) to utilise scientific data generated by the laboratories of NAS for generation, publication of scientific articles and annual laboratory reports.

11/1/2019

....

Author name: Ms Tersia De Beer
Designation: Scientist Production
Date: 11 January 2019

IT IS RECOMMENDED THAT DEPUTY DIRECTOR-GENERAL: AGRICULTURAL PRODUCTION, HEALTH AND FOOD SAFETY (APHFS) GRANT APPROVAL TO THE SUB-DIRECTORATE: NATIONAL ANALYTICAL SERVICES (NAS) TO UTILISE SCIENTIFIC DATA GENERATED BY THE LABORATORIES OF NAS FOR GENERATION, PUBLICATION OF SCIENTIFIC ARTICLES AND ANNUAL LABORATORY REPORTS.

....

Name:	Mr. B. M. Makhafola
Designation:	Director: Food Safety and Quality Assurance
Date:	10/8/2019

IT IS RECOMMENDED THAT DEPUTY DIRECTOR-GENERAL: AGRICULTURAL PRODUCTION, HEALTH AND FOOD SAFETY (APHFS) GRANT APPROVAL TO THE SUB-DIRECTORATE: NATIONAL ANALYTICAL SERVICES (NAS) TO UTILISE SCIENTIFIC DATA GENERATED BY THE LABORATORIES OF NAS FOR GENERATION, PUBLICATION OF SCIENTIFIC ARTICLES AND ANNUAL LABORATORY REPORTS.

Recommendation/s supported YES ☑ NO ☐

…………………………………………………………………………

Name: Mr. D. Serage

Designation: Chief Director: Inspection and Quarantine Services

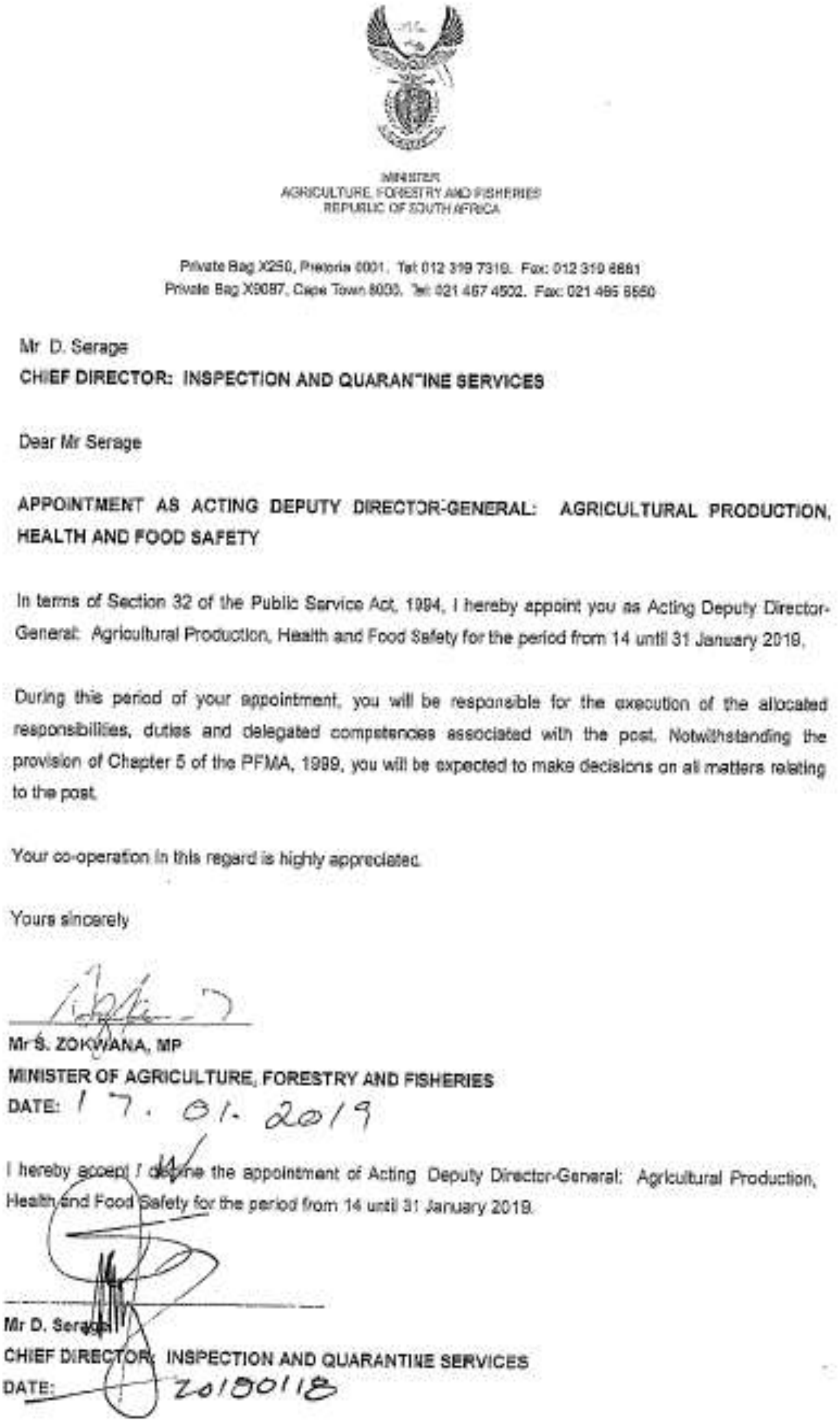
Date: 2019/01/14

Recommendation/s approved YES ☑ NO ☐

Name: Mr D. Serage
~~Mr. R. M. Ramasodi~~

Designation: Deputy Director-General: Agricultural Production, Health and Food Safety (Acting)

Date: 2019/01/22



MINISTER
AGRICULTURE, FORESTRY AND FISHERIES
REPUBLIC OF SOUTH AFRICA

Private Bag X250, Pretoria 0001. Tel: 012 319 7319. Fax: 012 319 6681
Private Bag X9087, Cape Town 8000. Tel: 021 467 4502. Fax: 021 465 6550

Mr D. Serage
CHIEF DIRECTOR: INSPECTION AND QUARANTINE SERVICES

Dear Mr Serage

APPOINTMENT AS ACTING DEPUTY DIRECTOR-GENERAL: AGRICULTURAL PRODUCTION, HEALTH AND FOOD SAFETY

In terms of Section 32 of the Public Service Act, 1994, I hereby appoint you as Acting Deputy Director-General: Agricultural Production, Health and Food Safety for the period from 14 until 31 January 2019.

During this period of your appointment, you will be responsible for the execution of the allocated responsibilities, duties and delegated competences associated with the post. Notwithstanding the provision of Chapter 5 of the PFMA, 1999, you will be expected to make decisions on all matters relating to the post.

Your co-operation in this regard is highly appreciated.

Yours sincerely

Mr S. ZOKWANA, MP
MINISTER OF AGRICULTURE, FORESTRY AND FISHERIES
DATE: 17. 01. 2019

I hereby accept / ~~decline~~ the appointment of Acting Deputy Director-General: Agricultural Production, Health and Food Safety for the period from 14 until 31 January 2019.

Mr D. Serage
CHIEF DIRECTOR: INSPECTION AND QUARANTINE SERVICES
DATE: 20190118