THE CHEMICAL, MICROBIAL AND SENSORY EVALUATION OF LUCERNE (*Medicago sativa* L.) FOR HUMAN CONSUMPTION AND ACCEPTABILITY

by

ANNCHEN MIELMANN

Submitted in fulfillment of the requirements
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Faculty of Natural and Agricultural Sciences
University of the Free State
Bloemfontein, South Africa

Promoter: Prof. C.J. Hugo
Co-promoter: Dr. C. Bothma

June 2014

This thesis is dedicated to my parents,

Heinrich Hermann Johannes Mielmann & Martha Petronella Esterhuizen

DECLARATION A

I declare that the thesis hereby submitted by me for the Philosophiae Doctor Food Science degree in the Faculty of Natural and Agricultural Science at the University of the Free State is my own independent work and has not previously been submitted to any other university, faculty or department, and that the copyright resides with the University of the Free State.

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A. Mielmann

June 2014

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UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS
UV

Research Division
Internal Post Box G40
☎(051) 4052812
Fax (051) 4444359

E-mail address: StraussHS@ufs.ac.za

Ms H Strauss/hv

2012-11-08

REC Reference nr 230408-011
IRB nr 00006240

MS A MIELMANN
c/o PROF CJ HUGO
DEPT OF MICROBIAL, BIOCHEMICAL AND
FOOD BIOTECHNOLOGY
AGRICULTURAL BUILDING 7.G15
FACULTY OF NATURAL & AGRICULTURAL SCIENCES
UFS

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cc **Prof CJ Hugo**

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(Language and style used in this dissertation are in accordance with the requirements of *Food Quality and Preference*)

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LIST OF ABBREVIATIONS

AA	-	Amino acid
AAS	-	Amino acid score
ADF	-	Acid Detergent Fibre
AHC	-	Agglomerate Hierarchical Clustering
Ala	-	Alanine
ALP	-	Alfalfa Leaf Protein
am	-	Anti meridiem
ANF	-	Anti-nutritional Factors
ANOVA	-	Analysis of Variance
ANU	-	Attribute Not Used
AOAC	-	Official Methods of Analysis
APC	-	Alfalfa Protein Concentrate
APC/d	-	Alfalfa Protein Concentrate per day
ARC	-	Agricultural Research Council
Arg	-	Arginine
ASM	-	Analytical Standard Method
Asp	-	Aspartic acid
ASTM	-	American Society of Testing and Materials
β	-	Beta (Standardised coefficient)
B	-	Boron
B	-	Unstandardized coefficient
B.C.	-	Before Christ
BFAP	-	Bureau for Food and Agricultural Policy
°C	-	Degrees Celsius
Ca	-	Calcium
cfu	-	Colony forming units
cfu/g	-	Colony forming units per gram
CHO	-	Carbohydrates
$C_8H_{10}N_4O_2$	-	Crystallised caffeine (monohydrate)
Cl	-	Chloride
cm	-	Centimetre
CNS	-	Central Nervous System

CO_2	-	Carbon dioxide
Corr	-	Correlation
CP	-	Crude Protein
Cu	-	Copper
Cys	-	Cysteine
d	-	Days(s)
DA	-	Descriptive Analysis
DM	-	Dry Matter
DM/ha	-	Dry matter per hectare
DNA	-	Deoxyribonucleic acid
EFSA	-	European Food Safety Authority
e.g.	-	For example
et al.	-	Et alia (and others)
etc.	-	Etcetera
EU	-	European Union
FAO	-	Food and Agriculture Organization
Fe	-	Iron
$FeSO_4$	-	Ferrous sulphate
Fig.	-	Figure
FS	-	Free State
FSP	-	Free State Province
g	-	Gram
GC	-	Gas Chromatography
g/d	-	Gram per day
GDA	-	Generic Descriptive Analysis
GDP	-	Gross Domestic Product
g/kg	-	Gram per kilogram
g/l	-	Gram per litre
Glu	-	Glutamic acid
Gly	-	Glycine
GM	-	Genetically Modified
GRAS	-	Generally Recognized as Safe
H	-	Hour(s)
H	-	Hypothesis

Ha	-	Hectare
His	-	Histidine
HLP	-	Hyperlipoproteinemia
HO-Pro	-	HO-Proline
HPLC	-	High-Performance Liquid Chromatography
HQ	-	High Quality
HRT	-	Hormone Replacement Therapy
H_2SO_4	-	Sulphuric acid
IAT	-	Implicit Association Test
ICMSF	-	International Commission on Microbiological Specifications for Foods
ICP-MS	-	Inductively coupled plasma-mass spectrometry
IDF	-	Intermediate Degradable Protein
i.e.	-	That is
IEC	-	International Electrotechnical Commission
IFT	-	Institute of Food Technologists
IgE	-	Immunoglobulin E
IGP	-	Intermittent Galvanostatic Polarisation
IITA	-	International Institute of Tropical Agriculture
IL	-	Illinois
Ile	-	Isoleucine
Inc.	-	Incorporated
INR	-	International Normalized Ratio
ISO	-	International Organization for Standardization
K	-	Potassium
KCl	-	Potassium chloride
KCl/ha	-	Potassium chloride per hectare
kg	-	Kilogram
kg/ha	-	Kilogram per hectare
km/h	-	Kilometre per hour
L	-	Linnaeus
LAA	-	Limiting amino acid
LAB	-	Lactic Acid Bacteria
Leu	-	Leucine

LiBr	-	Lithium bromide
LiCl	-	Lithium chloride
LOQ	-	Limit of quantification
LSD	-	Least Significant Difference
Lys	-	Lysine
M	-	Mean
M	-	Molar
MAYA	-	Most Advanced Yet Acceptable
MC	-	Mineral Concentration
ME	-	Metabolisable Energy
Met	-	Methionine
Mg	-	Magnesium
mg	-	Milligram
mg/g	-	Milligram per gram
mg/kg	-	Milligram per kilogram
min	-	Minute(s)
MJ/kg	-	Megajoules per kilogram
ml	-	Millilitre
Mn	-	Manganese
Mm	-	Millimetres
MRS	-	De Man, Rogosa and Sharpe
MUG	-	Methylumbelliferyl-β-D-glucuronide
n	-	Frequency
N	-	Nitrogen
n	-	Sample size
Na	-	Sodium
NaCl	-	Sodium chloride
NCSS	-	Number Cruncher Statistical Systems
ND	-	Not Determined
NDF	-	Neutral Detergent Fibre
NLO	-	National Lucerne Organization
nm	-	Nanometre
NMR	-	Nuclear magnetic resonance
No.	-	Number

NO_2	-	Nitrogen dioxide
NS	-	Not Significant
NSA	-	Not Statistically Analyzed
NZFSA	-	New Zealand Food Safety Authority
O_2	-	Oxygen
OM	-	Organic Matter
P	-	Phosphorous
PBPM	-	Pioneer Brand Products Manual
PCA	-	Principle Component Analysis
PDPNA	-	Panel on Dietetic Products, Nutrition and Allergies
PE	-	Petroleum Ether
Phe	-	Phenylalanine
PM	-	Preference Mapping
Pm	-	Post meridiem
Pro	-	Proline
Pty Ltd	-	Proprietary Limited
QDA	-	Quantitative Descriptive Analysis
r	-	Correlation coefficient
R	-	South African Rand
R^2	-	Square Root
RBC	-	Rose Bengal Chloramphenicol
S	-	Sulphur
SA	-	South Africa
SADAFF	-	South African Department of Agriculture, Forestry and Fisheries
SADoH	-	South African Department of Health
‘SAS’	-	‘SA Standard' (Lucerne cultivar)
‘SASP’	-	‘SA Standard plain’
‘SASS’	-	‘SA Standard stew’
SB	-	Spinach Beet
SBP	-	Spinach Beet Plain
SBS	-	Spinach Beet Stew
SD	-	Standard Deviation
SE	-	Standard Error
sec	-	Second(s)

Ser	-	Serine
SLE	-	Systemic Lupus Erythematosus
SLP	-	Soluble Leaf Protein
SPC	-	Standard Plate Count
SPCA	-	Standard Plate Count Agar
spp.	-	Species
SPSS	-	Statistical Package for the Social Sciences
SSA	-	Statistics South Africa
SSETA	-	Services Sector Education and Training Authority
t	-	T-test
TBC	-	Total Bacterial Count
Thr	-	Threonine
TLC	-	Thin Layer Chromatography
TPB	-	Theory of Planned Behaviour
TSS	-	Total Soluble Solids
Tyr	-	Tyrosine
UFS	-	University of the Free State
US	-	United States
USA	-	United States of America
Val	-	Valine
VRB	-	Violet Red Bile
‘WL525’	-	‘WL525’ (Lucerne cultivar)
‘WL525P’	-	‘WL525 Plain’
‘WL525S’	-	‘WL525 Stew’
‘WL711’	-	‘WL711’ (Lucerne cultivar)
‘WL711P’	-	‘WL711 Plain’
‘WL711S’	-	‘WL711 Stew’
Zn	-	Zinc
%	-	Percentage
µg/d	-	Microgram per day
µg/g	-	Microgram per gram

CHAPTER 1
INTRODUCTION

Over 7 000 plant species have been cultivated for food, yet it is estimated that 90% of the world's dietary energy supply is obtained from only 30 species. Thousands of plant species, and many more varieties, fall into a category defined as underutilized or neglected crops. From the nutrition perspective, a myriad of different food species, and varieties within species, are consumed in traditional diets, providing energy and nutrients, and thus contributing to food and nutrition security. Due to the lack of professional attention to biodiversity and underutilized foods in South Africa (SA), large gaps exist about the composition and dietary contribution of these foods to human nutrition. This has been an area neglected or regarded as unimportant by compilers of food composition databases and investigators involved in designing and executing food consumption surveys. Therefore, the real and potential contribution of these foods to nutrition and health improvements cannot be judged. Data on nutrients, bioactive non-nutrients and anti-nutrients are lacking for many food species and are even fewer for varieties, cultivars and breeds within species (Burlingame, Charrondiere, & Mouille, 2009).

Over the past few years, interest has grown about the health benefits of lucerne (*Medicago sativa* L.). Lucerne is the most widely spread (over 32 million ha worldwide) and one of the oldest cultivated legume forages in the world (Li, Su, & Yuan, 2007). Recently, with the increase in the demand for green food, people are paying more attention to the utilization of lucerne, because of its high nutritional contents (15 – 20% crude protein) (Colas, Doumeng, Pontalier, & Rigal, 2013; Lamsal, Koegel, & Gunasekaran, 2007). Lucerne is of immense importance for both developed and lesser developed countries, as it contains a high amount of protein and the yield of protein per unit area is greater than any other known conventional crop (Sen, Makkar, & Becker, 1998).

South Africa has an estimated population of 50.6 million people (SSA, 2011), of which 79% are Black Africans. The majority of SA households live in poverty, with a limited variety of foods (mainly staples) available in homes. In essence, most SA children consume a diet low in energy, and poor in protein quality and micronutrient density, while children from urban areas are increasingly overweight (Labadarios et al., 2001). Relieving the environmental pressure of food production for a growing world population – which might approach 9 billion by 2050 – is in different ways a challenge for all nations (United Nations Population Division [UNPD], 2010). To sustain this population growth over the next 30 years, it would be necessary to produce more foodstuffs throughout the world, than over the whole of the last 10 000 years (Kern, 2002). In developing countries, such as SA, food security is still the main issue, and it may take precedence over environmental impacts. In addition, much depends on the technological feasibility and the societal acceptability of potential solutions, such as a partial diet shift from animal protein to plant protein (Aiking & De Boer, 2004).

When striving for sustainable food production and consumption systems, an analysis of dietary proteins from various sources (i.e. meat, dairy and plant) is an excellent starting point (De Boer, Helms, & Aiking, 2006). It is argued that a transition, towards more plant protein based diets, would simultaneously benefit the conservation of biodiversity, land, water, energy, climate, human health and animal welfare. A trend reversal towards diets containing less animal protein and more plant protein, in Western countries, seems not just strongly recommendable, but inevitable (Aiking, 2010). Nearly four fifths of all protein food sources in the developing countries are derived from plants, but less than half of all protein sources are utilized in the developed countries (Grigg, 1995). Furthermore, it is estimated that, on a national scale, about a quarter of the world population consumes an unnecessarily high amount of animal protein and, as a consequence, indirectly devours a high amount of cereals. In theory, a reduction of animal protein in these diets would reduce this inefficient cereal demand. The resources, thus spared in the production of animal feeds, could then be used for the production of food for the growing population and the conservation of natural resources (Helms, 2004).

Even per capita, the world now produces 40% more food than 40 years ago. However, in the next 40 years, another 70% more is required (Aiking, 2010). Over the next decade, the growth in the consumption of chicken meat is projected to increase by 48%. The total consumption of chicken meat is projected to reach almost 2.4 million tons by 2020. Beef consumption is expected to grow by 23% and mutton consumption by 20%. Pork consumption is projected to grow by 46% until 2020 (BFAP, 2012). By the year 2020, the worlds' protein supplementation will have to double. What is striking is that Africa, as a continent, does not play a role or make a contribution towards protein supplementation in the world. The main issues affecting future growth trends in SA are thought to be population growth and food supply. Africa is the only continent where population growth rates (3.1%) have not yet started to decline. There has been an increase in fat (3.2%), and meat and egg consumption (2.8%), and a decrease in sugar consumption (4.2%). Black and coloured children also have a lower mean protein and fat intake. More recently, it was also reported that urban black female students consumed significantly more sugar and confectionary, and significantly less legumes and maize meal than their rural counterparts (Steyn, Abercrombie, & Labadarios, 2001).

The development of protein rich lucerne products that are tasteless will have no value for consumers. Sensory science has proven successful in research and development, and quality assurance in the food and beverage industries (Meilgaard, Civille, & Carr, 1999; Moskowitz, Beckley, & Resurreccion, 2006; Stone & Sidel, 1993). The potential of using sensory evaluation, to link recipe development to marketing, has also been recognized. Sensory science thus links the chain through the chemical-sensory descriptive interface and the sensory-descriptive-affective-behaviour interface (O'Mahony, 1995).

In the food industry, sensory analysis is used to establish differences, and to characterize and measure sensory attributes of products. It also establishes whether product differences are noticeable to consumers and whether these differences are acceptable or unacceptable to the target consumer group (Lyon, Francombe, Hasdell, & Lawson, 1992). Understanding development and variations in taste, which will occur during the development of lucerne products, is an important tool in defining consumers' expectations of taste. An increasingly important aspect of sensory analysis will be the evaluation of the relationship between preference or acceptability, and the descriptive sensory attributes of the lucerne. This allows the product developer to concentrate on the attributes and combination of attributes, which are likely to result in the so-called ideal product for a particular segment of the consuming population (Baker, 1988).

Therefore, it is becoming a reality that SA consumers need more plant protein legumes, such as lucerne, that can play a major role in consumers' diets and help to maintain health and prevent disease (e.g. malnutrition). The aim of this study was to investigate, by means of chemical, microbial and sensory methods, the feasibility of the introduction of lucerne into the diet of consumers as an alternative protein source, and to determine the knowledge and attitude of consumers towards lucerne.

The following hypotheses were formulated:

i. lucerne will indicate nutritional potential for human consumption and a potential cultivar for future research will be determined;
ii. lucerne could have a similar taste profile to spinach beet (SB) (*Beta vulgaris* var. *cicla* L.; a familiar leafy vegetable as control);
iii. the method of preparation and addition of ingredients will play an important role to make lucerne more acceptable for consumers;

iv. the descriptive attributes for lucerne will relate to consumer acceptability and will assist in the development and improvement of an acceptable lucerne product for SA consumers;

v. consumers' knowledge of lucerne differs from different demographic backgrounds that will indicate a need to inform consumers on the benefits of consuming lucerne; and

vi. specific variables such as health benefits, food safety risk, sensory qualities and synonyms of will affect consumers' attitude towards lucerne.

Hypothesis i) will be tested in Chapter 3; hypothesis ii) and iii) will be tested in Chapter 4; hypothesis iv) will be tested in Chapter 5 and hypothesis v) and vi) will be tested in Chapter 6.

The first objective of this study was to investigate the chemical (degrees Brix, macro- and micro-minerals, protein, amino acids, dry matter, moisture, ash, fat, fibre, carbohydrates and energy content) composition and microbial content, and shelf-life of three lucerne cultivars and compare it to SB, to determine which lucerne cultivar to use for future research in food product development.

The second objective was to determine the sensory profile and consumer acceptability of lucerne. Firstly, the sensory attributes of three lucerne cultivars were determined, by using generic descriptive analysis (GDA). Secondly, consumers' acceptance of lucerne was determined. This test included the same lucerne cultivars and one SB cultivar, which were evaluated for the degree of liking for aroma, taste, mouthfeel and overall acceptability. For this study, it was hypothesized that lucerne could have a similar taste profile as SB.

The third objective was to determine how the descriptive attributes for the three lucerne cultivars and one SB cultivar related to consumer acceptability, by using external preference mapping (PM). As the relationship between consumer acceptability and descriptive sensory attributes of lucerne cultivars have never been studied in SA, it was found necessary to perform PM on all three lucerne cultivars, in order to acquire an objective characterization of lucerne's sensory attributes. Furthermore, the results obtained from the lucerne cultivars will assist in the development and improvement of an acceptable lucerne product for SA consumers.

The fourth objective was to determine consumers' knowledge about and attitudes towards lucerne. Firstly, consumers' knowledge from different demographic groups was investigated. Secondly, the role of health benefits, food safety risks, sensory qualities and synonyms on attitudes towards lucerne, were investigated. Thirdly, consumers' attitudes towards lucerne were determined, by means of thematic analysis (a qualitative measure).

To the author's knowledge, the uniqueness of this study lies in the fact that lucerne (which is mainly used for animal feed) has not recently been investigated as an alternative protein source for human consumption in SA. Nor have the sensory properties or attitudinal behaviour of human consumers towards lucerne been investigated. Furthermore, no research was undertaken to raise the awareness of the potential utilisation of lucerne for human consumption, which could contribute to food security and human nutrition for sustainable development in SA.

Ethical approval for the study was obtained from the Ethics Committee of the Faculty of Health, University of the Free State, Bloemfontein (ECUFS NR: 183/2012; REC reference nr: 230408-011) and all ethical measures were practically applied.

CHAPTER 2
LITERATURE REVIEW

A part of this literature review (see annexure 1) has been published as: Mielmann, A. (2013). The utilisation of lucerne (*Medicago sativa*) – A Review. *British Food Journal*, *115 (4)*, 590–600.

2.1 Introduction

To sustain South Africa's (SA) population growth in the future, it is necessary to produce more protein based foodstuffs. The effective utilization of nonconventional foods should be more widely researched in SA, due to the shortage of conventional feedstuffs. Presently, SA does not contribute to research in protein supplementation (Laula, 2010).

Interest in how to effectively utilize lucerne as food for humans is growing (Gault et al., 1995). Lucerne or alfalfa (*Medicago sativa* L.) is an herbaceous forage legume and are a valuable source of proteins, essential amino acids, vitamins, minerals and dietary fibres. The promotion of lucerne leafy greens as an underutilised food source for human consumption should be considered, in order to increase the variety of plant sources available to consumers, and at the same time, to expand the utilization of the lucerne plant. As the consumption of lucerne could improve the protein intake of consumers, role-players in food-based programmes in SA can promote the cultivation and use of lucerne in rural and urban communities, in an effort to reduce malnutrition.

This literature review focuses on three major components, namely: 1) the nutritional and microbial composition of lucerne; 2) sensory analysis; and 3) consumer attitudes. These three components are intertwined and can, therefore, not be addressed in isolation. The first component of the research seeks to propose lucerne as a potential vegetable by discussing the chemical composition, protein applications, safety aspects and microbial diversity of lucerne. As lucerne cultivars are mainly researched for animal grazing purposes only, it is necessary to investigate the nutritional and microbial composition of different lucerne cultivars and to compare them to another known vegetable, such as Swiss chard or spinach beet (*Beta vulgaris* var. *cicla* L.), also referred to as "spinach" by most people in SA (SADAFF, 2013). This will allow for an investigation into the potential of lucerne for human consumption and will identify the best lucerne cultivar for future research. Certain barriers such as affordability, availability, household, taste preference, family influences and cultural beliefs must be addressed to ensure everlasting success of lucerne in the market.

The second component of this research establishes the need to sensorily evaluate lucerne, as preparing lucerne products that no human wants to consume, would be meaningless. It is argued that understanding the development and variations in taste through sensory methods, which will occur during the development of lucerne products, is an important tool in defining consumers' expectations of taste, as well as the success of the final product. The last component focuses on consumer behaviour, namely attitudes, since these are important predictors of both consumer food choice and behaviour, and needs investigation.

2.2 Background on lucerne

Lucerne (*Medicago sativa* L.) is a drought-resistant, hardy, perennial (Gault et al., 1995), herbaceous forage legume (Lenné & Wood, 2004), which can improve the barren and arid land (Hao et al., 2008; Li et al., 2007), and is recognised as the longest growing plant used in animal feed (NLO, 2010). The oldest recorded reference to date indicated that lucerne was used as forage more than 3 300 years ago. The Persians apparently grew this plant around 490 B.C. for horse and cattle feed (Scholtz, 2008). Lucerne is characterized by a stout taproot, purple flowers and spiral shaped pods (Figures 2.1 & 2.2). As the plant develops, a crown is formed, from which as many as twenty stems develop, normally reaching a height of 400 – 600 mm. The stems and leaves constitute the forage, which makes lucerne so valuable to farming. If the top growth is not removed and the plant is allowed to mature, it dies and new growth points develop from the crown. This happens between three to ten times per season, depending on temperature and available soil moisture (Dickinson et al., 2010).

Different names for this legume forage include alfalfa, lucerne, buffalo herb, Chilean clover, father of all foods and purple medic (Aganga & Tshwenyane, 2003). In the principle areas, namely Asia, Europe (Aganga & Tshwenyane, 2003), SA, Australia, New Zealand, and North and South America (NLO, 2010), the plant is known either as "alfalfa" or as "lucerne". The word "alfalfa" is of Arabic origin and means "the best fodder" (Scholtz, 2008). The name 'lucerne', variously spelled as luzern, lucern and lucerne, may have a much more modern derivation than the word alfalfa. Botanists use the 1753 classification of Linnaeus and common lucerne is known as *Medicago sativa* L. (NLO, 2010).

Fig. 2.1. The leaves, flowers and side-shoots of *Medicago sativa* L. carried on the stems.

Fig. 2.2. The purple flower of *Medicago sativa* L.

Lucerne hay, often called the ‘queen of forages’, is considered by the SA animal feed manufacturing industry as an important source of roughage for livestock (Scholtz, 2008). The current area, planted with lucerne for hay production in SA, is estimated to be between 208 000 ha and 240 000 ha, resulting in an average annual hay production of approximately 3.8 million tons, of which about 90% is under irrigation (Grönum et al., 2000). Lucerne was brought from France to the Cape Colony in SA around 1850, where it soon became important to the large ostrich farms. When the area of ostrich farming declined, lucerne remained and has since become widely grown on irrigated land throughout SA (Scholtz, 2008).

According to Goławska, Łukasik, Wójcicka and Sytykiewicz (2012), the high nutritional quality of lucerne is due to its substantial content of high quality protein and carbohydrates (Tharanathan & Mahadevamma, 2003). Lucerne contains between 15 – 22% crude protein (CP) on a dry matter (DM) basis, as well as all the macro- and trace minerals, and all the fat- and water- soluble vitamins (Scholtz, 2008), making it suitable as feed for monogastrics, such as poultry, swine and horses (Adapa, Schoenau, Tabil, Sokhansanj, & Singh, 2007). Lucerne also makes an excellent complement for grains and other forages (e.g. clover) in dairy diets (Martin & Mertens, 2005). However, only 60% of the protein in lucerne can be digested by animals (Thacker & Haq, 2009). Nitrate accumulation in forage is at a maximum under cool, cloudy conditions, which decreases photosynthesis and the reduction of nitrate to amino acids. Accordingly, the sunny and longer day length found in SA could explain the higher percentage of true protein that has been reported (Scholtz, 2008).

Apart from its uses to make hay and silage, lucerne also has numerous applications in the agricultural sector. Lucerne is an outstanding legume for grazing, because of its high yield, quality and wide adaptability to different climates and soil types (NLO, 2010). It can be grown as a cover crop and often increases the yield of succeeding crops such as potatoes, rice, cucumbers, lettuce and tomatoes. Lucerne extracts can produce antibacterial activity against Gram-positive bacteria. Seeds yield 8.5 – 11% of a drying oil, which is suitable for making paints and varnishes. Seed screenings are ground and used to a limited extent in feeds for ruminants. Lucerne is an excellent pasture for hogs, cattle and sheep, often mixed with grass. Supplement feeding of grain for dairy cows, sheep and fattening cattle reduces bloating and balances the high protein level of the lucerne pastures with energy, and extends the usefulness of the pasture (Aganga & Tshwenyane, 2003). Processed lucerne products, such as dehydrated pellets and cubes, have been used in ruminant rations (Mustafa, Christensen, & McKinnon, 2001).

For grazing purposes, lucerne is grown under irrigation or it is cultivated on dry land in the summer rainfall areas in SA. In addition to having the highest yield potential of all legumes cultivated in SA, lucerne thrives on a wide range of soils and climatic conditions, but it requires good drainage for optimal production. Dormant lucerne can survive for several days in standing water, but in the growing season it begins to die off after a few days. Lucerne is also very sensitive to acid soils and if the pH (KCl) decreases below 5.0, the growth vigour declines. Although lucerne is reasonably resistant to brack conditions, production is adversely affected if the pH (KCl) rises above 6.5. Because of its strong and deep taproot system, lucerne is particularly resistant to drought. Although it is by nature not productive during serious drought conditions, dry land lucerne recovers quickly after rain. It can be successfully cultivated in areas where the summer rainfall is as low as 400 mm (Dickinson et al., 2010).

March, April and May are the best months to sow lucerne. Cool, cloudy and moist conditions are favourable for its establishment. Earlier establishment can fail due to high temperatures. Although establishment in spring is not generally recommended, it should be done earlier rather than later, i.e. from mid-August to mid-September. The pH, phosphorus (P) and potassium (K) levels must also be corrected before establishment. Seedlings can survive light frost and can be considered safe from frost after the four-leaf stage. As with all pastures, a well-prepared, fine and weed-free seedbed is a necessity. Preparation of the soil must begin no later than January, as under dry land conditions, moisture must be accumulated in the subsoil before establishment. Because soil fertility and fertilization largely determine the success of lucerne production, it is necessary to follow optimal fertilization practices. Many soils are acidic and inherently deficient in one or more essential plant nutrients. The application of lime and fertilizers is necessary in most cases for its successful cultivation (Dickinson et al., 2010).

The following recommendations are made when cutting lucerne (Dickinson et al., 2010):

i. it should be cut in dry sunny weather, because if lucerne gets wet, losses in quantity and quality are inevitably high;
ii. it should be cut before regrowth from the crown begins, as loss of leaf is high when cut too late, while yield from the next cutting will also be adversely affected; and
iii. any handling should be done only early in the morning or late in the afternoon, when the dew or moisture is high, to limit leaf loss.

2.3 Cultivars

The most commonly-grown lucerne cultivar in SA is ‘SA Standard’. This cultivar has been consistently selected, since the seed of ordinary *M. sativa* was first imported in 1861. Over the years, many cultivars have been admitted to this country from overseas. In general, these cultivars have a higher yield than that of ‘SA Standard’. They also have an added advantage that they are very leafy and slow to flower; however, these cultivars, which have mainly been bred for haymaking purposes, are not as tolerant to grazing as ‘SA Standard’. ‘Baronet’ is recommended for grazing under dry land conditions, while ‘Pierce’ can be regarded as dual purpose lucerne. The cultivars ‘C.U.F. 101’ and ‘Granada’ is also known in SA (Dickinson et al., 2010).

Lucerne is classified according to its dormancy status. The scale used to classify this status ranges from 1 = highly dormant (adapted to very cold areas) to 11 = non-dormant (adapted to warm favourable conditions, highly productive and suitable for 8 – 10 harvests per year). For the purpose of this study, the researchers focused on three cultivars, namely ‘SA Standard’, ‘WL 525 HQ’ and ‘WL 711’ (see Table 2.1), as these cultivars have good leaf to stem proportion. All cultivars showed good field tolerance to disease, insects and nematode reaction. Eromosele, Arogundade, Eromosele and Ademuyiwa (2008) state that research efforts are now focusing on the identification and evaluation of the potential of underutilised forage legume sources, such as lucerne, with good nutritional qualities, that are well adapted to adverse environmental conditions (high seed yield and pest and diseases resistant). Relevant information on each cultivar is provided in Table 2.2.

For many years producers have been dependent on ‘SA Standard’, the result of natural selection between ‘Chinese’, ‘Hunters River’ and ‘Provence’ – cultivars that were imported during the 1930s. ‘SA Standard’ was preferred over the small number of other available cultivars, because of its high grazing tolerance, drought resistance and high tolerance to root and crown diseases. Furthermore, ‘SA Standard’ also has the advantage of being cultivated for SA’s specific climatic conditions. In general, imported cultivars only last around 4 – 6 years, while ‘SA Standard’ has a life expectancy of up to 12 years on dry lands and 15 years under irrigation. ‘SA Standard’ and ‘SA Select’ accounts for more than 50% of the local lucerne market (Dickinson et al., 2010).

Cultivar ‘WL 525 HQ’ is a consistently high-yielding, high-quality lucerne cultivar. Dairy producers and hay-makers appreciate the exceptional speed of recovery after cutting, superior stand persistence and strong package of pest and disease resistance traits. Its winter activity offers opportune grazing when feed is often scarce. ‘WL 525 HQ’ is the recommended variety for irrigating dairy producers, specialist hay producers and those looking to feed lambs or cattle (Kyneton, 2013).

Table 2.1.

Lucerne (*Medicago sativa* L.) cultivars available for purchase in South Africa (K2 Agri, 2011; Pannar, 2011).

Cultivar	Dormancy status	Description
WL 357 HQ	5.5 (winter dormant)	Good leaf to stem proportion; good tolerance to *Fusarium* wilt and *Phytophthora* root rot; suitable for dry land grazing conditions.
SA Select	6 (semi-winter active)	Good leaf to stem proportion; generally acceptable disease tolerance; good field tolerance to aphids; multipurpose cultivar; dry land and/or irrigation.
SA Standard	6 (semi-winter active)	Common dry land and irrigation cultivar; relatively good leaf to stem proportion; generally acceptable disease, insect and nematode tolerance.
WL 414	6.5 (semi-winter active)	Multipurpose cultivar; dry land and/or irrigation; good leaf to stem proportion; good field tolerance to disease, insect and nematode reaction.
KKS 3864	7 (semi-winter active)	Multipurpose irrigation cultivar; good leaf to stem proportion; good field tolerance to disease, insect and nematode reaction.
SARDI 7	7 (intermediate)	A very promising variety suitable for hay production or grazing; relatively fine stalks and holds its bottom leaves very well; high yield potential.
KKS 9595	7.5 (semi-winter active)	Very good irrigation grazing cultivar; good leaf to stem proportion; good field tolerance to disease, insect and nematode reaction.
PAN 4884	8 (semi-winter active)	Offers good protection against diseases, insects and eelworm; very responsive to inputs that enhance yield; more suitable for hay-making in more stressful environments; use under irrigation for optimal production.
WL 525 HQ	8 (winter active)	Excellent irrigation hay cultivar for dairy farmers; good leaf to stem proportion; good field tolerance to disease, insect and nematode reaction.
PAN 4961	9 (strong non-dormant)	Very non-dormant; high yielding, bred exclusively for hay production. Upright growth habit.
Robusta	9 (winter active)	Excellent multipurpose irrigation cultivar - good performance under stress conditions; good leaf to stem proportion; good field tolerance to disease, insect and nematode reaction.
WL 612	9 (winter active)	Very good grazing under irrigation; good leaf to stem proportion; good field tolerance to disease, insect and nematode reaction.
WL 625 HQ	9 (winter active)	Excellent irrigation hay cultivar; excellent leaf to stem proportion; good field tolerance to disease, insect and nematode reaction.
WL 711	10 (winter active)	High yielding irrigation hay cultivar; excellent leaf to stem proportion; good field tolerance to disease, insect and nematode reaction.

Table 2.2.

Lucerne cultivars used in this research study (data from Dickinson et al., 2010).

	'SA Standard'	'WL 525 HQ'	'WL 711'
Description	Common dry land and irrigation cultivar; relatively good leaf to stem proportion; generally acceptable disease, insect and nematode tolerance	Excellent irrigation hay cultivar for dairy farmers; good leaf to stem proportion; good field tolerance to disease, insect and nematode reaction	High yielding irrigation hay cultivar; excellent leaf to stem proportion; good field tolerance to disease, insect and nematode reaction
Type	Semi-winter active	Winter-active	Winter-active
Dormancy status	6	8	10
Purpose	Common dry land and irrigation cultivar	Excellent hay cultivar for dairy farmers	High-yielding hay cultivar
Cultivation	Dry land and/or irrigation	Irrigation	Irrigation
Plant Characteristics			
Crown position	Intermediate	Above ground	Above ground
Stem	Coarse to medium	Thin-medium	Medium-relatively thin
Fibre	Medium to high	Medium	Medium-relatively low
Leaf	Fairly leafy-tri-leaf type	Fairly leafy-tri-leaf type	Leafy-tri-leafy type
Leaf colour	Medium green	Deep green	Deep green
Hay composition	Relatively good leaf to stem proportion	Good leaf to stem proportion	Excellent leaf to stem proportion
Sowing rate (Recommended seed density)	12 – 15 kg/ha (Dry land) & 20 – 25 kg/ha (Irrigation)	20 – 25 kg/ha (Irrigation)	20 – 25 kg/ha (Irrigation)
Disease reaction	Generally acceptable disease tolerance	Good tolerance to *Fusarium* wilt and *Phytophthora* root rot	Good tolerance to *Fusarium* wilt and *Phytophthora* root rot
Insect and nematode reaction	Generally acceptable insect and nematode tolerance	Good field tolerance to aphids, earth louse and root knot eelworm	Good field tolerance to aphids, earth louse and root knot eelworm

Cultivar ‘WL 711’ is the first dormancy lucerne variety available in SA. It is highly active in winter and has rapid regrowth following each cutting. It performs outstandingly in warm production areas with long day lengths and does better in sandy soils. It delivers outstanding yields of high-quality hay (Hitterstay, 2007).

2.4 Lucerne for human consumption

Lucerne is the most efficient source of protein that can be grown in most parts of the temperate world. Moreover, it is becoming clear that much of the world can no longer afford the inefficiency of converting plants to meat. Lucerne protein has more potential for alleviating world hunger and concomitantly reducing the ecological costs of agriculture, than any other plant (Munro & Small, 1997).

Munro and Small (1997) stated that the earliest documented use of the lucerne genus was as human food. Flannery as cited by Munro and Small (1997) describes that in a south-western Iranian farming village, dated 7 500 – 5 600 B.C., wild lucerne seeds, and possibly also wild lucerne stems were consumed, probably in a mixed gruel. He noted that the high-protein wild legumes collected locally, probably contributed to a diet better than that available in modern Iranian villages. Lucerne has been used for human consumption in Europe in times of shortage. For example, in Spain during the Civil War, lucerne served as the basis of many dishes such as lucerne soup. The mature plant is coarse and has a grassy flavour. The young leaves, which are very nutritious and an excellent source of protein and vitamins (Larkcom, 2007), have been consumed as a vegetable in countries like China (Davidson, 2006). Lucerne meal was incorporated into a special "cereal mixture" and used in the feeding of small children (Bolton, 1962; Levy & Fox, 1935).

Recently, with the increase in the demand for green food, people are paying more attention to the utilization of lucerne in the food industry, because of its high nutritional contents (Hao et al., 2008; Lamsal et al., 2007). Green lucerne is actually used to a small extent as a human food in the form of vegetables in parts of Russia and America, while it is occasionally used as a substitute for spinach in SA (Levy & Fox, 1935).

According to Blumenthal (as cited by Bolton, 1962), lucerne flour is rich in alkaline minerals and is invaluable for acidosis. Bolton (1962) sampled a prepared breakfast food that contained a portion of dehydrated lucerne meal, and found that the flavour was not unpleasant, although the lucerne could be detected by taste. The product did, however, not become popular enough to be stocked on grocery shelves. In order to improve the nutritional values and functional properties of flour, lucerne could also be utilized to increase the protein, dietary fibre, and mineral and vitamin content of wheat flour (Hao et al., 2008).

Haggart (as cited by Bolton, 1962) obtained manufacturer's samples of a blended flour, 'tea' and 'coffee', made from lucerne, together with samples of lucerne cookies (cakes), crackers (biscuits), and candy (sweets). The crackers and cookies were palatable, but the candy had such a pronounced lucerne flavour that it was not enjoyed. The previously-mentioned flour was mixed with wheat-flour and used to make griddle cakes, muffins, biscuits, doughnuts and cake, which were sampled by consumers. Consumers agreed that the greenish colour was undesirable in a prepared food, but that the griddle cakes, doughnuts, muffins and cake were very palatable and tasty. Some consumers did, however, object to the flavour of lucerne in the biscuits. The 'tea' prepared from the dried leaves was very much like an ordinary herbal tea, for which a taste might be cultivated and the 'coffee' made from baked leaves, was as agreeable as any similar cereal product. In all cases the boiling liquid smelled strongly of lucerne (Bolton, 1962). Cotto (2010) speculated that initially many people will not like its taste, or it might also create the sense that it is burning the tongue tip. He recommended that one should persevere and continue to use lucerne as it is definitely an acquired taste.

Stramesi and Falabella (as cited by Bolton, 1962) stressed the nutritive value of the leaves and suggested using only tender leaves from the first crop, harvested before the plants flower. Stems should be avoided, because of their high fibre content. As reported by them, 50 g/d of lucerne could be eaten easily, and this quantity was used as a basis for the preparation of a number of dishes. The lucerne should be boiled in boiling water for 10 min. to remove unpleasant flavours. Thereafter it could be passed through a sieve or chopped before serving. After several experiments, Stramesi and Falabella suggested recipes such as lucerne salad, lucerne soup, lucerne tortilla, lucerne stew and lucerne pudding. It was found that the flavour of lucerne meal was not unpleasant, although the lucerne could be detected by taste.

The use of germinated lucerne seeds originated in Far East countries and has recently spread to the Western world, where they are considered fashionable and healthy ingredients (Goławska et al., 2012; Peñas, Gómez, Frías, & Vidal-Valverde, 2009). Lucerne is one of the most popular sprouts available on European markets. The sprouts are consumed either raw or slightly cooked in salads and sandwiches or as decorative appetizers. In SA, they are sold at convenience stores separately or in combination with other ingredients like chick peas. It is well known that the germination process improves the nutritional value of the sprouts, compared to unprocessed seeds. This germination process either increases digestibility by reducing anti-nutritional factors or increases compounds with antioxidant activity (Peñas et al., 2009).

Reports claim that the inclusion of forage legumes in the daily diet has many beneficial physiological effects in controlling and preventing various metabolic diseases, such as diabetes mellitus and colon cancer. Currently, the role of forage legumes as therapeutic agents in the diets of persons with metabolic disorders, is gaining interest (Tharanathan & Mahadevamma, 2003). In Chinese and Hindu societies, physicians use young lucerne leaves to treat disorders related to the digestive tract, arthritis and water retention. These physicians also make a cooling poultice from the seeds for boils. In Colombia, lucerne leaves are used to treat coughs. Lucerne leaves may have a therapeutic effect on gastric ulcers and has been used in the treatment of kidney stones (Readers' Digest, 2006). Today, lucerne leaves is used in homeopathic preparations worldwide to treat anaemia, to increase appetite to contribute towards weight gain and to act as a diuretic for increased urination and bladder disorders (Foster & Johnson, 2006). However, claims of the efficacy of homeopathic practices beyond the placebo effect are unsupported by the collective weight of scientific and clinical evidence (Ernst, 2002).

Lucerne can help to reduce exhaustion and nervous agitation. Due to lucerne's phytoestrogen (isoflavones and coumarins) content, it is thought to regulate menstrual cycles and to stimulate milk flow in breastfeeding women. Experiments carried out by clinical nutritionists in 1982, demonstrated that eating lucerne helped to protect monkeys that were on a high-cholesterol diet from atherosclerosis. The experiments also proved the effectiveness of lucerne in decreasing blood-cholesterol levels. Lucerne seeds can be added to the diet to help normalize serum cholesterol concentrations in patients with type II hyperlipoproteinemia (HLP) (Mölgaard, Von Schenck, & Olsson, 1987).

Lucerne may also be used as a traditional plant treatment for diabetes. The administration of lucerne in the diet (62.5 g/kg) and drinking water (25 g/l) reduced the hyperglycaemia of streptozotocin-diabetic mice. The results demonstrated the presence of antihyperglycaemic, insulin-releasing and insulin-like activity in lucerne. Lucerne leaves are used traditionally as a tea to treat diabetes in SA. The use of lucerne as an antidiabetic agent in human subjects has, at least in part, been attributed to its relatively high manganese (Mn) content (Gray & Flatt, 1997).

2.4.1 Protein applications

According to Smith (1970), it has been suggested that lucerne be used as a high yielding source of protein for human consumption. Lucerne protein, which is extracted from the lucerne leaf, is a good source for the production of nutritious and functional food (NLO, 2010). Research has been conducted on preparation procedures, application, property and nutritional value of lucerne leaf protein. Using a sequential extraction procedure, proteins have been isolated from the dry powders of six one-year old and two more than one-year-old Australian lucerne herbages. Lucerne biomass has potential biotechnological importance in the production of low fibre, juice-derived co-products such as particulate (chloroplastic) protein concentrates, soluble protein concentrates, carotenoids, vitamins, minerals, growth factors, pharmaceutical agents, cosmetic products and transgenic enzymes (Sreenath, Koegel, Moldes, Jeffries, & Straub, 2001).

The novel food ingredient, alfalfa protein concentrate (APC), has long been recognized as a potential source of high-quality protein (45 – 60%) for human and animal consumption (D'Alvise, Lesueur-Lambert, Fertin, Dhulster, & Guillochon, 2000). The use of ACP in human food is limited by their negative sensory properties, which include dark colour due to polyphenols, granular texture, poor solubility and grassy taste (D'Alvise et al., 2000; Xie et al., 2008) possibly due to their saponin content (Thacker & Haq, 2009).

Food properties that may be influenced by APC include water holding capacity, emulsification, foaming, viscosity, gelation and texture (Lamsal et al., 2007). An APC dose of 10 g/d has been tested for its nutritional value in several clinical trials, in countries such as Peru, India and Congo. Alfalfa Protein Concentrate has been used since 1992 as a food supplement, to combat malnutrition in several non-EU countries and has been consumed in 20 countries throughout the world, with no harmful effects being reported by various national governmental organisations. The use of APC has also been authorised in the USA, Canada and Mexico (EFSA, 2009).

Lucerne products are available in drinks and chocolate bars, but mostly as food supplements (capsules, tablets, powder). A survey by the European Food Safety Authority

(EFSA) confirmed that lucerne is consumed as food supplements, as well as an ingredient in well-known foods, such as soups and salads. However, no quantitative data were available (EFSA, 2009).

In response to lucerne's decrease in competitiveness against rival products (such as oil seed cakes) and its falling subsidies, the lucerne industry had to diversify in Europe (Petin & Luzerne, 2010a). Leaf protein has also been recognized by the Food and Agriculture Organization (FAO) as a potential source of high-quality protein for human consumption, due to its abundance of source, nutritive value and absence of animal cholesterol (Xie et al., 2008).

In the FRALUPRO project, supported by the European Union, lucerne juice was fractionated to create nutritional and functional protein ingredients for the food and non-food industry (Petin & Luzerne, 2010a; 2010b). Through advanced technology (i.e. fractionation of lucerne, extracting of the protein) it could be demonstrated that lucerne contains a protein called Rubisco (ribulose 1, 5-bisphosphate carboxylase). Also known as Fraction-I protein, it accounts for up to 30 – 70 g/100 g of soluble lucerne leaf proteins (SLP) (Lamsal et al., 2007). Furthermore, it also accounts for approximately 2% of the total DM fraction of lucerne and helps plants to convert energy from the sun. It could profitably replace soy as a source of protein in food. At the moment, almost 80% of plant proteins in food come from soy, but none of them covers humans' nitrogen and amino acid requirements. In contrast, Rubisco contains all the essential amino acids which humans need and is closer to milk proteins. It also has foaming and emulsifying properties, which could have applications in foods, cosmetics and detergents. The production of plant proteins is known to be infinitely more profitable than the production of animal proteins, since the return is 10 – 100 times higher, depending on the plant (Petin & Luzerne, 2010a).

2.5 Safety of lucerne

It is very difficult to obtain scientific data for nearly all plant foods, which document their history of safe consumption, even though they may have been eaten for several hundreds of years (Knudsen, Søborg, Eriksen, Pilegaard, & Pedersen, 2008). There are certain precautions to be considered when consuming lucerne. Consumption should be avoided during pregnancy and breastfeeding. A physician should be consulted if using hormone replacement therapy (HRT). It should also be avoided in case of diabetes or systemic lupus erythematosus (SLE) and with the use of anticoagulants, such as warfarin or Heparin® (NLO, 2010). Large quantities of lucerne sprouts can cause a form of anaemia. In the USA, lucerne herb and seed are generally recognized as safe (GRAS) (EFSA, 2009); however, isolated cases of *Salmonella* and *Escherichia coli* contamination in lucerne sprouts have been reported, but no such cases have so far been reported in SA (NLO, 2010).

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies (PDPNA) was asked to deliver a scientific opinion on the safety of APC as food. In the report, the applicant proposed the use of APC as a food supplement with a recommended consumption of 10 g/d. The PDPNA concluded that the coumestrol, isoflavone and L-canavanine content of APC, as well as the use of APC as a food supplement at the proposed level of 10 g/d, present no safety concerns (EFSA, 2009).

Lucerne foliar extracts contain phytoestrogens (coumestrol and isoflavones), that are known to disturb the reproduction cycle in females. The total isoflavone (daidzein, genistein, glycitein) content of APC is 255 mg/kg, which is similar to the level of the total isoflavonoids present in soy products. The suggested consumption of 10 g APC/d leads to an intake of 2.6 mg of isoflavones, which is about 17% of the average total isoflavone intake via soy-based foods in the French adult population. Also, the coumestrol content of APC (78 mg/kg) is comparable to that of the main sources of coumestrol intake, namely fresh soy sprouts and split peas. The PDPNA noted that for adults and children weighing more than 32 kg, the amounts of coumestrol (0.8 mg) and isoflavones (2.6 mg), in the 10 g recommended daily dose of APC, did not exceed the maximum recommended safe levels of isoflavone consumption of 1 mg/kg of body weight per day. It was further concluded that the intake level of phytoestrogens in a 10 g daily dose of APC, is lower than that of other common food sources and therefore does not raise concern (EFSA, 2009).

L-canavanine, which is mainly present in the seeds and sprouts of most leguminous plants, has been suspected of being responsible for systemic lupus erythematosus (SLE) activation. However, the L-canavanine concentration of APC (4.3 mg/kg) is very low, compared to other common food sources, such as lentil flour (2 800 mg/kg) and onions (10 000 mg/kg). Moreover, APC did not lead to an anti-DNA (double stranded) antibody production (a SLE marker) in a mice study designed to investigate lupus. The PDPNA concluded that the L-canavanine content of APC is of no concern. An *in vivo* study showed ingestion of APC caused slight allergic manifestations in mice, sensitized to peanuts. Data are lacking on cross-reactivity with peanuts using immunoglobulin E (IgE) binding, skin prick testing, or double-blind placebo-controlled approaches. The PDPNA concluded that the risk of cross-reactivity in subjects, allergic to peanuts, cannot be excluded (EFSA, 2009).

Furthermore, the PDPNA noted that there were no sub-chronic, chronic, reproductive and developmental toxicological data available on APC. It was concluded that other toxicological data concerning the levels and effects of anti-nutrients and secondary metabolites (in particular L-canavanine and phytoestrogens) in APC, the information regarding nutritional effects in animal and human studies, and the history of its use as a food supplement without reported adverse effects, supported the safely use of APC (EFSA, 2009).

Lucerne should be avoided with the use of anticoagulants (NLO, 2010). Anticoagulant (or anti-clotting) medications are used to prevent blood clots or to stop an existing clot from getting larger. These drugs, sometimes referred to as “blood thinners”, include warfarin (Coumadin®), dalteparin (Fragmin®), danaparoid (Orgaran®), enoxaparin (Lovenox®), Heparin® (Hep-Lock, Hep-Pak, Hep-Pak CVC, Heparin Lock Flush) and tinzaparin (Innohep®). Anticoagulant medications are mostly used to prevent blood clots from forming after a surgical procedure and to reduce the risk of a stroke or heart attack (Medifast, 2008). People who take anticoagulant medications need to be aware of their intake of foods that are high in vitamin K. Lucerne is a valuable source of vitamin K (Aganga & Tshwenyane, 2003), a fat-soluble vitamin, which plays an important role in helping blood to clot. Anticoagulant medications work to decrease the activity of vitamin K in the body, and a controlled vitamin K intake is needed to help anticoagulant medications work as effectively as possible (Medifast, 2008). Oral anticoagulants exert their effect by blocking the utilization of vitamin K, yet little is known about the competitive aspects of their interaction with dietary vitamin K. Schurgers, Shearer, Hamulyák, Stöcklin and Vermeer (2004) carried out systematic dose-response studies in healthy volunteers, who had been stably anticoagulated and maintained on their individualized doses for 13 weeks. The response to vitamin K-rich food items was also tested. The short-lived response after meals of spinach and broccoli suggested an inefficient bioavailability from these two sources. It was concluded that short term variability in vitamin K1 intake is less important to fluctuations in the international normalized ratio (INR), than has been commonly assumed and that food supplements providing 100 μg/d vitamin K1 do not significantly interfere with oral anticoagulant therapy.

2.6 Nutritional composition of lucerne

Green forage of lucerne is reported to contain, 80% moisture, 5.2 g protein, 0.9 g fat, 3.5 g fibre and 2.4 g ash per 100 g edible portion. Lucerne whole meal and leaf meal are reported to contain, 276 and 322 kilojoules (kJ), 7.5% and 8.0% moisture, 16.0 g and 20.4 g protein, 2.5 g and 2.6 g fat, 27.3 g and 17.1 g fibre, 9.1 g and 11.5 g ash, respectively, per 100 g. Lucerne is a valuable source of vitamins A and E, and also contains ß-carotene, thiamine, riboflavin, niacin, pantothenic acid, biotin, folic acid, choline, inositol, pyridoxine, vitamin B12 and vitamin K (Aganga & Tshwenyane, 2003). It is also an outstandingly good source of vitamin C (1.78 mg/g) (Levy & Fox, 1935), but loses 80% of this upon drying (Aganga & Tshwenyane, 2003), while vitamins B and D are present only in low concentrations. The presence of vitamin D_2 and vitamin D_3 was also successfully demonstrated (Horst, Reinhardt, Russel, & Napoli, 1984). In addition, about 8 mg/g iron (Fe) is present – nearly double that, found in spinach (Levy & Fox, 1935). Scholtz, Van Der Merwe and Tylutki (2009) investigated the nutritive value of lucerne samples, representing the SA lucerne hay population and found that the CP consists of 76.9% true protein (average = 207 g/kg DM). The chemical composition of lucerne is presented in Table 2.3, while Table 2.4 shows the average nutrient levels in the top 150 mm, at vegetative growth stage.

Yari et al. (2012) investigated the nutrient profiles of lucerne harvested at different morphological stages of maturity and at different times during the day, in relation to the diet formulation for dairy cows. Lucerne was cut at the early bud, late bud and early flower stages, both in the afternoon (06:00 pm) and the next morning (06:00 am). With advancing maturity of lucerne, leaf content, leaf:stem ratio, calculated energy values, CP, *in situ* digestibility (especially at 12 and 36 h of incubation) and nitrogen (N) to energy ratios [organic matter (OM), carbohydrates (CHO)] decreased ($p<0.05$). On the other hand, neutral detergent fibre (NDF), acid detergent fibre (ADF), fibre associated CP and total CHO increased ($p<0.05$), with advancing maturity of lucerne. Protein and CHO fractions (defined conforming to the Cornell net carbohydrate and protein system), associated with different degradation characteristics, stayed consistent with advancing maturity, except for intermediate degradable protein (IDP), which decreased at the early flower stage, compared to the early and late bud stages. Lucerne harvested in the afternoon tended to have a higher leaf portion and leaf:stem ratio ($p=0.06$) and contained 13 g/kg more CHO (soluble carbohydrates) ($p<0.01$), 27 g/kg more IDP ($p=0.02$), and 0.33 MJ/kg more DM ($p<0.05$).

Table 2.3.

Chemical composition of lucerne (*Medicago sativa* L.).

Contents	Guerrero-Rodríguez, Revell, & Bellotti (2011)	Scholtz et al. (2009)
g/kg DM		
Ash	151.6	129.7
Crude protein	297.8	207
Soluble protein	-	62
Acid detergent fibre - crude protein	-	17
Acid detergent fibre	-	332.2
Neutral detergent fibre	-	440.6
Lignin	-	73.5
Cellulose	-	258.7
Hemicellulose	-	108.4
Na	0.9	-
Cl	6.3	10.7
Ca	34.3	13.5
P	2.9	-
K	28	25.3
Mg	5.6	-
S	4.4	-
mg/kg DM		
Zn	126.3	36
Fe	91.5	874
Mn	52.2	-
B	69.3	-
Cu	-	7

Maturity of the lucerne plant seems to have the greatest influence on the variation of chemical parameters. It is generally known that the quality of lucerne hay drops as the plant matures. This drop is largely due to the increasing stem:leaf ratio and to an increase in the fibre content of the stems (Scholtz, 2008). As predicted by Smith (1970), the highest concentrations of nutrition constituents occur in the leaves. During the early flowering stage, the leaves contain a greater concentration of digestible nutrients, proteins, fats, fibre, total non-structural carbohydrates and other micro-nutrients, than the stems. In return, stems have more sugars, fibre and K, hence it is clear that lucerne leaves contain more nutrients than the stems (Scholtz, 2008).

Table 2.4.

Average nutrient levels in lucerne (top 150 mm at a vegetative growth stage) (PBPM, 2011).

Nutrient	Unit	Normal range
Nitrogen	%	4.5 – 5.0
Phosphorus	%	0.26 – 0.70
Potassium	%	2.5 – 3.8
Sulphur	%	0.26 – 0.50
Calcium	%	0.51 – 3.00
Magnesium	%	0.31 – 1.00
Sodium	%	0.00 – 0.05
Iron	µg/g	30 – 250
Manganese	µg/g	30 – 100
Zinc	µg/g	20 – 70
Copper	µg/g	10 – 30
Boron	µg/g	30 – 80
Molybdenum	µg/g	0.90 – 2.00

Lucerne quality (nutritive value) varies considerably and is influenced by general factors, such as harvesting at specific physiological stages, climatic factors, edaphic factors such as soil conditions, storage practice, disease and insects, weeds, lucerne cultivar, moisture content during storage, water supply and fertilization (Scholtz, 2008).

2.7 Microbial diversity

Production practices, growth conditions and the location of the edible part during growth can affect vegetables' microbial status, which, in turn, may affect the health of consumers. Contamination of vegetables may take place at all stages during pre-harvest techniques (Halablab, Sheet, & Holail, 2011). Possible sources of these pathogens are soil, faeces (manure, both of human and animal origin), water (irrigation, cleaning), ice, animals (including insects and birds), handling of the products, harvesting, processing, equipment and transportation. In order to determine the microbiological quality of vegetables, microbiological analysis, such as total aerobic flora, total coliforms, thermo-tolerant coliforms, anaerobic sulphite-reducing bacteria, *Staphylococcus aureus*, fungi and *Salmonella* spp., could be investigated (Adjrah et al., 2011). Oladele and Olakunle (2011) stated that the spoilage of green leafy vegetables is due to the activity of microorganisms - the conditions, favourable for their proliferation, being moisture and temperature.

The microorganisms normally present on the surface of raw fruits and vegetables may consist of chance contaminants from the soil or dust (Halablab et al., 2011). These include bacteria or fungi that have grown and colonized, by utilizing nutrients exuded from plant tissues. Among the groups of bacteria commonly found on plant vegetation are coliforms or faecal coliforms, such as *Klebsiella* and *Enterobacter*. Epiphytic micro-organisms, naturally present on forage crops, are responsible for silage fermentation and also influence the effectiveness of silage bacterial inoculation. The microorganisms of greatest concern in silage preservation are lactic acid bacteria, (e.g. lactobacilli), Enterobacteriaceae, yeasts and moulds, and clostridial spores. Because silage is preserved by lactic acid fermentation, only homo-fermentative bacteria (e.g. pediococci, streptococci and some species of lactobacilli) are beneficial - others either cause inefficient fermentation or lead to silage deterioration. Epiphytic microorganisms are affected by forage species, stage of maturity, weather, mowing, field-wilting and the chopping process. Lin et al. (1992) identified and enumerated the microorganisms on lucerne, as well as the factors that influence them. They found that Enterobacteriaceae were the predominant microorganisms on standing lucerne and weather conditions (particularly temperature) during the growing season significantly influenced the epiphytic microorganisms. However, neither cutting nor maturity had a significant effect on the epiphytic microorganisms on standing lucerne, and wilting lucerne, after mowing, also did not affect the population. In fact, the chopping process tended to increase the numbers of epiphytic microorganisms.

Plate counts of aerobic mesophilic microorganisms found in food is one of the most used microbiological indicators for food quality. These organisms reflect the exposure of the sample to any contamination and the existence of favourable conditions for the multiplication of microorganisms in general. Coliforms are facultative anaerobic, Gram-negative, non-spore forming rods, which ferment lactose with gas formation within 48 h, when grown in lactose broth at 35°C. They are commonly used bacterial indicators of the sanitary quality of foods and water. *Escherichia coli* forms part of the coliform group and is one of the species associated with faecal contamination. It is naturally found in the intestines of humans and warm-blooded animals (Halablab et al., 2011).

Human and animal enteric pathogens (except soil-borne spore formers such as *Clostridium perfringens* and *Bacillus cereus*) are usually absent from fresh vegetables at harvest, unless they have been fertilized with human or animal wastes or irrigated with water containing such wastes (ICMSF, 1986). The FAO (2008) reported an *E. coli* 0157:H7 spinach outbreak in the USA in 2006, where contamination was caused by swine waste (Jay et al., 2007). Between 1973 and 2006, 502 (4.8%) outbreaks, 18 242 (6.5%) illnesses and 15 (4.0%) deaths were associated with "leafy greens" described as lettuce, cabbage, mescalin mix, spinach or a salad item containing one or more of these leafy vegetables (FAO, 2008). Barth, Hankinson, Zhuang and Breidt (2009) investigated the microbial spoilage of leafy vegetables. Spinach tested negative for bacterial vegetable pathogens (*Pseudomonas*, *Erwinia*, *Xanthomonas*, *Bacillus*, *Clostridium* and lactic acid bacteria) and fungal vegetable pathogens (*Geotrichum*, *Rhizopus*, *Phytophthora*, *Fusarium*, *Pythium*, *Alternaria*, *Colletotrichum*, *Botrytis* and *Sclerotinia*).

Most studies investigated the microbiological hazards in lucerne sprouts and not in lucerne leaves. A large number of viable microorganisms are known to develop on lucerne sprouts during the first days of the sprouting process. The microorganisms may occasionally contain pathogenic bacteria that could be the source of outbreaks, such as *Bacillus cereus*, *Klebsiella pneumonia*, different *Salmonella* species or enterohaemorrhagic *Escherichia coli*. Outbreaks of salmonellosis have been linked to contaminated seeds from which, provided with a suitable environment during germination, *Salmonella* grew to high numbers (Palmai & Buchanan, 2002). In 1995, an outbreak of *Salmonella enterica* infections occurred in the USA, because of the consumption of contaminated lucerne sprouts. Outbreaks of food poisoning caused by *Escherichia coli* O157:H7 and infection by other *Salmonella* spp. have also been associated with the consumption of raw lucerne sprouts (Soylemez, Brashears, Smith, & Cuppett, 2001).

All foodstuffs, however, should not contain microorganisms at levels which may cause harm to humans upon consumption. This is one of the regulations of section 2(1)(b)(i) of the Foodstuffs, Cosmetics and Disinfectants Act (SA, 1972). It must be borne in mind that the presence of microorganisms in foods is not necessarily an indicator of a hazard to the consumer. Plants and animals form the major origin of the foods which are eaten and these sources are naturally associated with microorganisms, which imply that foods will be associated with microorganisms (SADoH, 2000). Table 2.5 shows the proposed microbiological specifications to be used as guidelines for raw vegetables.

Table 2.5.

Proposed microbiological specifications for raw vegetables and raw fruits (SADoH, 2000).

Food type	Analysis	Limits
Raw vegetables and raw fruits, including fresh fruit salad, salad dressing and peanut butter	Coliform count	<200 cfu/g
	Yeast and mould count	<100 000 cfu/g
	Escherichia coli	0 cfu/g
	Salmonella species	0 cfu/25 g

2.8 Sensory research as scientific discipline

Sensory evaluation is used to evoke, measure, analyse and interpret reactions to the characteristics of foods, as they are perceived by the sense of sight, smell, taste and hearing (Stone & Sidel, 2004). According to Brody and Lord (2008), the primary role of sensory analysis is to provide information on how the sensory characteristics of products, ingredients or other related information and services, relate to consumer liking and the quality characteristics as perceived by the consumer. Other benefits, such as health aspects, may enhance the perceived value of food, but they are useless if the sensory quality fails to attract consumers. Sensory analysis of food and data on consumer responses are therefore fundamental information for predicting the success of a product (MacFie, 2007).

Sensory research originated in the late 1940s and 1950s, and scoring procedures were already in use in the 1940s, with the emphasis on paired procedures for assessing product differences and preferences. In the 1950s, universities in the US initiated independent courses in sensory evaluation (Stone & Sidel, 2004). Discrimination test procedures were evaluated by Boggs and Hansen (1949), Girardot, Peryam and Shapiro (1952), and Pfaffman, Schlosberg and Cornsweet (1954). In 1957 the Flavour Profile method was introduced, which provided a focal point for sensory evaluation and created new interest in the discipline. More research and development were stimulated into all aspects of the sensory process, with rank-order procedures and hedonic scales becoming more common in the mid- to late 1950s. Further interest in descriptive methods developed as a result of the growth in new products and competition in the marketplace for products with unique sensory properties, as well as by advances in measurement and improved data processing systems (Stone & Sidel, 2004).

The Quantitative Descriptive Analysis (QDA) method, developed in the 1970's, was a substantive departure from existing methods. The approach was primarily behavioural in orientation, with a consensus approach to language development (no expectation that all subjects will be equally sensitive), use of replication for assessing subject and attribute sensitivity, and for identifying specific product differences and defined statistical analyses. This method would be more than a simple rephrasing of test questions or use of a particular scale. After a long and somewhat difficult development, sensory evaluation has emerged as a distinct, recognized scientific specialty (Stone & Sidel, 2004).

Sensory science has proven successful in research, development and quality assurance in the food and beverage industries. Furthermore, it links the chain through the chemical-sensory descriptive interface and the sensory-descriptive-affective-behaviour interface (O'Mahony, 1995). In the food industry, sensory analysis is used to establish differences, and to characterize and measure sensory attributes of products or to establish whether product differences are noticeable to consumers and whether these are acceptable or unacceptable to the target consumer group (Moskowitz et al., 2006). The various applications of sensory analysis include: shelf-life studies; product matching; product specifications and quality assurance; product reformulation; testing for taint potential; and determining product acceptability (Lyon et al., 1992).

In sensory science, analytical sensory evaluation and consumer sensory evaluation are used. Analytical sensory evaluation uses trained judges (humans) as analytical instruments to measure the sensory characteristics of foods. The consumer sensory evaluation consists of consumers who are users of the product, but who may not know about its composition or the nuances that it can assume. These two panels serve entirely different purposes in the product development cycle (Moskowitz, 1984).

The validity and reliability of the sensory testing methodologies are extremely important if test results are to be used as a basis for business decisions (Brody & Lord, 2008). The selection of all sensory tests should be based on the objectives for the study. If the objective is to quantify consumer acceptance, then a consumer affective test is appropriate; if the objective is to describe the characteristics of a product, then the test should include a descriptive sensory analysis of product attributes (Brody & Lord, 2008). As reported by Stone and Sidel (2004), reliance on a single method is particularly risky, because it leads one to modify problems to fit the method and to overlook basic sensory procedures and practices.

An increasingly important aspect of sensory analysis is the evaluation of the relationship between preference or acceptability and the sensory characteristics of the samples. This allows the product developer to concentrate on the attributes and combination of attributes, which are likely to result in the so-called ideal product for a particular segment of the consuming population (Baker, 1988).

2.8.1 Sensory acceptance

Consumer evaluation concerns itself with testing certain products, by using untrained people who are or will become the ultimate users of the product. This kind of testing is necessary throughout the various stages in the food product cycle, including the development of the food product itself, product maintenance, product improvement, and optimization and assessment of market potential (Brody & Lord, 2008). As proposed by Schifferstein (2010), sensory analysis is mainly focused with describing the perceptual characteristics of the product, whereas consumer studies often evaluate whether products are pleasant, attractive or beautiful. Moskowitz et al. (2006) noted that traditional consumer science deals with decision making, not products. The new concept science would begin by identifying consumer needs and incorporating them into product concepts. All consumers have different likes and dislikes with respect to food and their likes change with time and with product usage. Thus, a consumer may be able to say whether he or she likes a product, but is not able to say why, or to provide a sensory interpretation of his or her tastes (Le Calvé, 2000).

Consumer sensory research can identify consumer needs which are not being met by currently available food products, anticipate future needs, aid in the identification of new markets and business opportunities, and help to prevent the costly errors of producing and marketing food products to an ideal target group which does not exist. Consumer tests are conducted to obtain market and product information, by determining the personal reaction (acceptance and/or preference) of current or potential consumers to a product, a product idea or specific product characteristics (Meilgaard, Civille, & Carr, 1991).

Consumer affective tests are conducted throughout the different phases in the product development process. There are two approaches to consumer acceptance testing: the measurement of preference; and the measurement of acceptance (Jellinek, 1964). Consumer acceptance of a food may be defined as: (1) an experience, or feature of experience, characterized by a positive attitude towards the food; and/or (2) actual utilization (such as purchase or eating) of food by consumers (Moskowitz et al., 2006). In addition, Leighton (2007) reported that food acceptance refers to the palatability, hedonic tone, liking or disliking, food taste preference and pleasantness accompanied by the consumption of foods. Acceptance tests, therefore, measure consumer acceptance or liking of a product (Meilgaard et al., 1999; Moskowitz et al., 2006; Stone & Sidel, 1993). Sensory acceptance tests are conducted during product development for development guidance, to screen products, and to identify those products that are significantly disliked and those that match or exceed a specified target product for acceptance. These tests also indicate the acceptance of a product without the package, price and label. The implicit goal behind any and all sensory evaluation efforts in the food industry is to enhance the quality and improve the appearance, flavour and texture, as perceived by consumers, in order to influence their food choices (translated into purchase) at the point of sale (Brody & Lord, 2008).

Moskowitz (2000) listed the three major reasons for obtaining consumer input product acceptance - food manufacturers realize that consumer acceptance, first and foremost, determines product success; speed to market - consumer input shortens development time; and the cost of product testing is minimized (Brody & Lord, 2008).

In contrast, preference tests measure liking of one product, in a set, over another (Brody & Lord, 2008; Stone & Sidel, 1993). Preference tests refer to all affective tests, based on a measurement of preference (i.e. choice) or a measurement from which relative preference may be determined (IFT, 1981). Preference, in its most classical treatment by experts in the food industry, was defined in three different ways: (1) an expression of higher degree or liking; (2) choice of one object over others; and (3) psychological continuum of affectivity (pleasantness/unpleasantness), upon which such choices are based (Amerine, Pangborn, & Roessler, 1965). It may include the choice of one sample over another, a ranked order of liking, or an expression of opinion on a hedonic (like/dislike) scale. With the proliferation of sensory research procedures, however, it is probably most productive to limit preference to choice-based studies, where respondents choose the sample that they prefer, using the criteria imposed by the researcher. Preference methods can be used to determine differences in preference, but not differences of products - discrimination tests should be used for this purpose (Moskowitz et al., 2006; Stone & Sidel, 1993).

Taste preferences in food often reflect a consumer's social and cultural origins, social ambitions, as well as cultural capital acquired. From ancient days to the modern world of today, food and taste preferences have been closely linked to cultural development. Often, with affluence, consumers move from satisfying basic physiological needs, to fulfilling social and psychological needs which are shaped by the sub-cultures to which they belong (Wright, Nancarrow, & Kwok, 2001).

The methods, most frequently used to determine preference and quantify acceptance, are the paired preference tests for preference (selection of one product over another) and tests employing the nine-point hedonic scale, respectively. Acceptance measurements can be made on single products and do not require comparison with another product. The questions asked during acceptance tests are "How much do you like the product?" (Stone & Sidel, 1993) or "How acceptable is the product?" (Brody & Lord, 2008; Meilgaard et al., 1999; Stone & Sidel, 1993).

2.8.2 Descriptive analysis

Descriptive analysis is defined as a methodology that provides word descriptions (attributes) of products that also includes the intensities or strengths for each of those attributes. Descriptive analysis has successfully been used to obtain detailed descriptions of the aroma, flavour and oral texture of foods and beverages. Samples are evaluated for a number of attributes by a trained panel (Lea, Næs, & Rodbotten, 1998; Leighton, 2007). Trained panellists assess overall quality, according to the conformity of an array of sensory descriptors to predetermined standards, and consequently provide valuable information to the food industry in the definition of the sensory profiles for their products (Resano, Sanjuán, Cilla, Roncalés, & Albisu, 2010). This provides an objective measure of the sensory quality of food products (Álvarez & Blanco, 2000). The sensory profiles obtained can be used in product development and manufacturing, and are of particular use to nutritionists and food scientists who are interested in the development of food and beverage products (Woods, 1998). These include activities such as the development of standards for quality control purposes, documenting product attributes before consumer testing, to help select attributes for inclusion in the consumer questionnaire, helping to explain results of consumer tests, as well as tracking sensory changes and reformulating existing products due to ingredient changes (Lawless & Heymann, 1998; Leighton, Schönfeldt, & Kruger, 2008; Meilgaard et al., 1999).

To improve the assessment of sensory properties, techniques were developed for detailed sensory analysis of food products. For instance, on the basis of terminology expertise and a linguistic analysis of definitions used in sensory analysis, Giboreau et al. (2007) proposed a set of guidelines to obtain more accurate definitions for sensory descriptors. In addition, to avoid the figurative use of sensory descriptors, participants could be trained to comprehend the exact meanings of the sensory descriptors, e.g. Quantitative Descriptive Analyais (QDA). In the training, they could be provided with reference samples to define the exact quality the descriptor refers to and to pinpoint the various anchor points on the sensory-perceptual scales (e.g. the Sensory Spectrum approach). In addition, visual pictograms may be provided for further clarification of the verbal descriptors. All these procedures could help in preserving the sensory meaning of the verbal anchors (Schifferstein, 2010).

Descriptive analysis describes all sensations perceived, when evaluating a sample by different categories such as appearance, aroma, flavour or taste, texture and aftertaste (Majchrzak, Lahm, & Dürrschmid, 2010). A sample can be an existing product (currently in the marketplace), an ingredient, an idea, or an entirely new product for which there is no existing competition (Stone & Sidel, 2004). Every category is described by certain descriptors and definitions, which are discussed and agreed upon by the panellists during training sessions with a number of samples (Granitto, Gasperi, Biasioli, Trainotti, & Furlanello, 2007; Majchrzak et al., 2010). Data, generated by a trained panel using DA, can be used to interpret consumers' hedonic responses to the same samples (like or dislike), allowing impressions developed during DA to be related to views that lead consumers to accept or reject a product (Leighton, 2007).

Descriptive analysis methods, such as QDA, flavour profiling and the Spectrum method has been used for the sensory evaluation of plant sources. Most of these methods, however, imply the use of trained and experienced assessors, whose training involves a lot of time and expenditures. The generated terminology also tends to be complex and scientifically orientated. Developing a lexicon for the sensory testing of a product is a critical step in the research process. Descriptive analysis is a time-consuming process, however, it can provide invaluable information regarding potential rejects and reasons for rejection by consumers (Van Oirschot, Reeds, & Aked, 2003). Therefore, it is necessary to investigate the relation between consumer acceptance and descriptive sensory attributes. Obviously, many other factors also determine the success of a new vegetable cultivar, such as lucerne, in the market place, such as the DM content (texture), the yield (economic factor) or pest resistance. Product expectation, generated by marketing messages through the media or packaging, could further influence the success of a product in the market place (Leighton et al., 2008; Wright et al., 2001).

2.8.2.1 Descriptive sensory studies performed with plant sources

Table 2.6 provides a list of plant species where DA was used as the sensory evaluation method. All these studies aimed to improve consumer-focused knowledge about the quality factors that determine preference for these plant sources. A list of sensory criteria was developed to compare the sensory properties of the plant sources and to explain the preference of consumers. Although plant species like nopalitos (young cactus pear cladodes), cow pea leaves and lucerne are widely consumed, their sensory characteristics have received little attention. For this reason, these studies were performed to determine which sensory attributes were most important for quality purposes. Nyambaka and Ryley (2004) used DA to characterize the sensory properties of dehydrated cowpea leaves. A sensory evaluation system was developed by Blanda et al. (2010), in which a DA scheme was used, to define the sensory attributes of boiled potato slices. Voon et al. (2007) described the surface colour, odour, flavour and texture of durian fruit during storage, using DA with 16 descriptors, while Leighton (2007) used DA to describe the complete profile of different sweet potato cultivars.

2.9 Relationship between consumer acceptance and descriptive sensory attributes

According to McEwan, Earthy and Ducher (1998), consumers are clear on which products they like and dislike, but they are not always able to describe specifically why they like or dislike a product. Any explanation, given by consumers, also tends to be of a hedonic nature and not descriptive. The provision of attribute rating scales for consumers also holds various risks: different consumers will interpret attributes in different ways; consumers are open to biases in the use of response scales; and consumers will utilise these attributes as criteria in the provision of an overall preference or acceptance rating. It is, therefore, necessary to relate external information about the products to consumer preference ratings, in order to understand what attributes of a product drive preference. Using sensory analysis and consumer information independently does not always enable the researcher to derive the most benefit from available resources. Sensory assessors are sometimes required to give preference or acceptability

Table 2.6.

Descriptive sensory studies on plant sources.

Plant source	Reference
Coconut (*Cocos nucifera* L.)	Gatchalian, De Leon, & Yano, 1993
Cooked rice	Yau & Huang, 1996
Sweet-corn products	Rodrigue, Guillet, Fortin, & Martin, 2000
Broccoli (*Brassica oleracea* L. var. *italica* cv. 'Marathon')	Jacobsson, Nielsen, Sjöholm, & Wendin, 2004
Cowpea leaves (*Vigna unguiculata*)	Nyambaka & Ryley, 2004
Tomatoes (*Lycopersicon esculentum* Mill.)	Krumbein, Peters, & Brückner, 2004
Nopalitos (*Opuntia* spp.)	Pérez-Cacho, Galán-Soldevilla, García, & Montes, 2006
Durian fruit (*Durio zibethinus* cv. D24)	Voon et al., 2007
Yams (*Dioscorea* spp.)	Nindjin et al., 2007
Sweet potato	Leighton et al., 2008
Blackberries (*Rosaceae*, genus *Rubus*)	Du, Kurnianta, McDaniel, Finn, & Qian, 2010
Boiled potatoes	Blanda et al., 2010
Monofloral citrus, rosemary, eucalyptus, lavender, thyme and heather honeys	Castro-Vázquez, Diaz-Maroto, Gonzalez-Vinas, & Perez-Coello, 2009
Potato flavour	Morris, Shepherd, Verrall, McNicol, & Taylor, 2010

information, and are unlikely to be representative of the target population, and, because of their training, are more perceptive than the average consumer. Likewise, consumers are frequently asked to give reasons for their judgements or descriptors, but while these can provide some useful information, they need to be interpreted with care. Consumer descriptors are rarely detailed enough or reproducible, and can therefore lead to misleading results, due to the difficulties encountered in the interpretation process. By using sensory and consumer techniques in conjunction, a more complete picture can be obtained (McEwan et al., 1998).

Multivariate techniques have been frequently applied to investigate complex consumer perceptions and their sensory drivers. More specifically, several studies have used a multivariate approach, combined with DA, to help interpret consumer perceptions of freshness in apples, strawberries and carrots. In addition, multivariate relationships between consumer hedonic response data and descriptive sensory data have been applied to predict consumer liking or preference to newly developed products (Heenan, Dufour, Hamid, Harvey, &

Delahunty, 2008). According to Krishnamurthy, Srivastava, Paton, Bell and Levy (2007), this type of integrated approach enables an alternative to expensive and time-dependant consumer testing of newly developed products.

2.9.1 Analysis of variance

Analysis of variance (ANOVA) is a common statistical test method used in DA and other sensory evaluation methods, to compare more than two products, by using scaled responses. This technique compares the means from several samples and tests, whether they are all (within the experimental error) the same or significantly different. Analysis of variance evaluates one dependent variable at a time (Magoro, 2007).

2.9.2 Correlation analysis

As reported by Meilgaard, Civille and Carr (2007), correlation analysis is used for measuring the strength but not the quantitative nature, of the linear relationship between two variables (e.g. consumer ratings and descriptive data from a trained panel, descriptive attribute ratings and instrumental measurements). The typical correlation statistic is the Pearson product moment correlation (Moskowitz et al., 2006). Correlation coefficient (r) summarizes the strength of the relationship between attributes and its value lies between -1 and +1. A negative value (-1) indicates a perfect inverse linear relationship between variables, whereby one variable decreases as the other variable increases. In the case of a perfect positive direct linear relationship, both variables either increase or decrease together. A strong correlation (near to 1) implies that the two variables co-vary to some degree, whereas a value near zero indicates that there is little linear relationship between the variables (Magoro, 2007). The analyst should always examine the scatterplots of the paired variables before deciding if the value of r is an adequate summary of the relationship. Care should be taken to ensure that the data, to which correlation analysis is applied, arise from a single population and not a blend of heterogeneous ones (Meilgaard et al., 2007).

2.9.3 Principle component analysis (PCA)

An initial correlation analysis might identify one or more groups of variables that are highly correlated with each other (and not highly correlated with variables from other groups). This suggests that variables in each group contain related information and that possibly a smaller number of latent variables would provide an adequate summary of the total variability. Principle component analysis is the statistical technique used to identify the smallest number of latent variables, called “principal components”, that explain the greatest amount of observed variability. It is often possible to explain as much as 75 – 90% of the total variability in a data set consisting of 25 – 30% variables, with as few as 2 – 3 principle components. Principle component analysis also visualizes the correlation structure of a group of multivariate observations and identifies the axis along which the maximum variability in the data occurs. This axis is called the first principle component (PC1) on the horizontal axis. The second principle component (PC2) is the vertical axis, along which the greatest amount of remaining variability lies, subject to the constraint that the axes must be perpendicular (at right angles) to each other (i.e. orthogonal or uncorrelated) (Meilgaard et al., 2007). As reported by Moskowitz et al. (2006), the sensory analyst uses PCA to reduce the number of variables (such as flavour and/or texture attributes, consumer acceptance attributes, physiochemical measurements, ingredient levels) to a smaller number of components, with little loss of information. Examination of the original variables that are grouped in each of the principle components, usually gives meaningful insight into the type of variation being explained by each of the principle components.

2.9.4 Preference mapping (PM)

Preference mapping may be useful in explaining the relationship between consumer acceptability and DA (Caspia, Coggins, Schilling, Yoon, & White, 2006). It is only the consumer who can realistically provide hedonic data, while a trained sensory panel is able to provide reliable descriptive information. By relating these two data sets, one compliments the other, thus maximising the available information (McEwan et al., 1998). Since many product characteristics are interrelated, this approach will enable the product developer to identify the various needs which should be adjusted, as well as indicate the effect other characteristics may have on liking. This approach also provides information on consumer segmentation, allowing the product developer to ‘target’ his/her product appropriately. While at first the approach may appear to require excessive resources, it may in fact reduce the overall input by scientifically designing the ideal product (McEwan, 1996). The procedure requires an objective characterization of the products’ sensory attributes, achieved by DA, which is then related to preference ratings for the product, obtained from a representative sample of consumers (Murray & Delahunty, 2000).

According to Næs, Brockhoff and Tomic (2010), the most important data set for PM is the matrix of consumer acceptance scores for all the products involved. This gives a data matrix, with dimensions equal to the number of samples (J) x the number of consumers (N). The sensory data set has the same number of rows (samples) as the consumer data set, but the columns correspond to the sensory attributes (*K*). The third data set, containing information about consumer demographics and attitudes has dimensions equal to the number of consumer attributes (*M*, attitudes, demographics) x the number of consumers.

Preference mapping can broadly be divided into internal and external PM. Internal PM uses consumer acceptance ratings to locate the products on the maps (Meilgaard et al., 2007). It also uses the hedonic scores of the consumer as the active variables and the sensory characteristics as supplementary variables. The goal is to achieve a consensus configuration of the stimuli based solely on consumer preference data, since a sample map and a consumer map are obtained, which correspond to the scores and loadings of the PCA. The joint plot shows the consumers as directions of increasing preference on the sample map, where moving in the direction of preference means samples become “more liked”, and moving in the opposite direction means samples become “less liked”. On the other hand, external PM uses sensory descriptive attribute ratings to locate the products on the maps. It is obtained from PCA-analysed DA data and then correlates each of the consumers to this PCA space by regression analysis (Moskowitz et al., 2006).

2.10 Linking sensory research and consumer behaviour

For most people food is, and has always been, a matter of pleasure. The hedonic characteristics of food - primarily taste, but also appearance and smell - thus constitute a central aspect for consumers and their food choices (Leighton, 2007). Already in 1989, Rose Marie Pangborn predicted that convincing academics and administrators of the importance of behavioural research (consumers) and its scientific value, was not easy (Pangborn, 1989). Many standard sensory methods were derived from or inspired by the field of psychophysics (e.g. Moskowitz, 2003), which typically employs experiments under strictly controlled laboratory conditions. However, this approach has its limitations for understanding food perception, because in everyday situations, food perception depends on the contextual conditions and the way the food is consumed, and there is a need to extend sensory investigations beyond the properties of the food itself (Schifferstein, 2010).

Hekkert and Schifferstein (2008) defined the subjective product experience as the awareness of the psychological effects elicited by the interaction with a food product, including the degree to which all the senses are stimulated, the meanings and values people attach to the food product, and the feelings and emotions that are elicited (Schifferstein, 2010). While the former perceptual part is generally regarded as largely affectively neutral, the latter part is laden with affect and is referred to as the aesthetic part of the food product experience. In both cases, the focus is very much object-oriented, because it is the object that is described as for example, heavy, shiny and attractive. In this case the object refers to a food product. However, if a human-centred focus is adopted, it is possible to look at the effects that interactions with food products have on their users. On the basis of what is perceived through the senses, people try to understand how a food product should be prepared correctly or consumed, and people attribute an array of expressive, symbolic or other connotative meanings to it. Furthermore, if using the food product is helpful (or obstructive) in reaching an important life goal, or if it concurs (or conflicts) with a personal standard, it is likely to result in an emotional reaction (Desmet & Hekkert, 2002; Schifferstein, 2010).

Aesthetic principles, based on meaningful properties, are not necessarily linked to specific object features and are, therefore, modality-independent. These include familiarity, novelty and challenge. First of all, people tend to prefer food products that they have encountered frequently (Zajonc, 1968). In addition, aesthetic appreciation is linearly related to the degree of prototypically - the extent to which a food product summarizes the information all foods of a particular product category have in common. Despite people's preferences for familiar food products (e.g. Swiss chard or spinach), they are also attracted to new, unusual and innovative food products (e.g. lucerne), partly to overcome boredom and saturation effects. The reconciliation of the tendency to look for the familiar and the novel is found in the MAYA (Most Advanced, Yet Acceptable) principle, which suggests that people prefer products with an optimal combination of typicality and novelty (Schifferstein, 2010).

When people encounter an unknown food product (e.g. lucerne), they instinctively try to determine its identity and its potential usefulness. They tend to explore the food product in order to determine what it is, what it is made of, how its "parts" are connected, and what can be done with it. Apart from information contained in the physical product, the knowledge a person has about other food products or related product categories is used in identifying the product. Product meaning has various layers, varying from what the food product is and does, and how it can be used, to the qualities it expresses. In addition, the food product conveys information on its user, its producer and possibly, its creator. It may reveal what it is composed of, how and where it was manufactured, the people who helped manufacture and transport it, and the impact of its production on the environment and society. All these different types of information can affect the way in which the food product is experienced and evaluated (Schifferstein, 2010).

In particular, consumers need to change their behaviour in order to adopt the product. Confronting consumers with unfamiliar products (e.g. lucerne) may lead to unrealistic situations and information that has limited predictive validity. After all, for new or unknown food products, consumers have less information in their memory to guide them and expressions of preference are often constructed at the time that the respondent is asked to give a judgement (Urala & Lähteenmäki, 2004).

Moskowitz, Beckley and Resurreccion (2006) noted that traditional consumer science deals with decision making and not products. The new concept science would begin by identifying consumer needs and incorporating them into food product concepts. MacFie (2007) pointed out that optimizing on emotions and on hedonics (liking) can lead to different outcomes and he questioned whether liking should always be the appropriate criterion. He suggested going beyond liking or emotion to a more holistic construct, such as "enjoyment of the total product experience". Interposed between the sensory attributes of food and behaviour are cognitive factors, such as expectation and attitude (MacFie, 2007). It is important to note that attitude and behaviour are separate concepts. Simply because an individual has a particular attitude about something, does not mean that the individual will act on the attitude (Blythe, 2008).

Urala and Lähteenmäki (2004) observed that the means-end chain analyses show that health-related attitudes have become a central and important aspect to many consumers. Consumers form preferences based on health-related attitudes, motivated by expectations of both a longer life and a higher quality of life, which has also been found in other studies of food-related attitudes. Furthermore, the issue of naturalness is quite visible in the hierarchical value maps, indicating that consumers attach increasing importance to the way food is produced (Urala & Lähteenmäki, 2004).

Preferences are based on the belief that by consuming a particular food the outcome will be positive. The positive outcomes include beliefs about foods that are healthy or unhealthy, foods that are appropriate for certain occasions or people, and those that are not (Conner, 1993). It is recognised that understanding consumer attitudes and behaviour is essential information, and ought to be known before one formulates a product, rather than manufacturing a product and looking to others (Stone & Sidel, 2004).

2.10.1 Defining consumer attitudes

According to Lake (2009), Hawkins and Mothersbaugh (2010), and Schiffman and Kanuk (2010), an attitude is a learned predisposition to behave in a consistently favourable or unfavourable way, with respect to a given object. Many definitions have been formulated to define attitudes. Table 2.7 provides definitions for ‘attitude’ from the 1950’s to the present. It could be concluded that an attitude is a psychological tendency that is expressed by evaluating a particular entity, with some degree of favour or disfavour (Eagly & Chaiken, 1995). An attitude is formed during the encounters a person has with an attitude-object. During these encounters, beliefs about the attitude-object are formed. An attitude consists of an organization of several beliefs, focused on a specific object or situation. Beliefs, in return, are defined as “salient information relevant to the behaviour” (Ajzen, 1991). An individual may hold several beliefs about an object and with each of these beliefs comes an evaluation (Dreezens, Martijn, Tenbült, Kok, & De Vries, 2005). An attitude object is any person, object, advertisement or issue to which there is an attitude. It also endures over time and must apply to many different situations and is not a momentary event. It is important for the researcher to understand how attitudes are formed and how they could be influenced, since this could help influence consumers’ decisions (Noel, 2009). All these beliefs and their evaluations together form an attitude (Dreezens et al., 2005). A belief can develop into an attitude, according to the strength of feeling involved. The stronger the belief, the stronger the attitude becomes and the more likely that it will affect behaviour. Attitudes also imply a sense of generalization and longevity (Wright, 2006).

In simpler terms, an attitude is a complex mental state involving what we know, our feelings, our values and predispositions to act in certain ways. According to Loudon and Della Bitta (1993), an attitude is “an enduring organization of motivational, emotional, perceptual and cognitive processes, with respect to some aspects of the individual’s world”. More specifically, an attitude refers to a predisposition to respond in a consistent or predictable manner to a stimulus (Evans et al., 2009). Attitudes are also relatively enduring overall evaluations of objects, products, services, issues, or people (Babin & Harris, 2009; Hoyer & MacInnes, 2004). They play a critical role in consumer behaviour and are especially important, because they motivate people to behave in relatively consistent ways. As reported by Babin and Haris (2009), consumers in general have positive attitudes toward products that provide value.

Table 2.7.

Summary of definitions for ‘attitude’ in use from 1957 to 2013.

Definition	Reference
The degree of positive or negative affect associated with some psychological object.	Edwards, 1957
An implicit drive producing a response, considered socially significant in the individual's society.	Freedman, Sears, & Carlsmith,1978
Bi-dimensional, because consumers purchase goods and services and perform consumption behaviour for two basic reasons, (1.) consummatory affective (hedonic) gratification (from sensory attributes), and (2.) instrumental, utilitarian reasons concerned with "expectations of consequences".	Batra & Ahtola, 1990
Do not consist of the behavioural responses themselves or their probability, but it is instead an intervening variable, occurring between the stimulus and the response, that can be inferred only from overt behaviour.	Reardon, 1991
A psychological tendency that is expressed by evaluating a particular entity with some degree of favour-disfavour, like-dislike, satisfaction-dissatisfaction, or good-bad polarity.	Eagly & Chaiken, 1993
An enduring organization of motivational, perceptual and cognitive process, with respect to some of the individual's world.	Loudon & Della Bitta, 1993
Represents what an individual knows about and how he feels, and intends to act towards an object or situation.	Hargie, Saunders, & Dickson, 1994
Characterized by a predisposition or state of readiness to act or react in a particular way to certain stimuli.	Chisnall, 1994
Is the result of the beliefs and feelings people have about themselves, about other people and about the tasks they are faced with.	Lamberton & Minor, 1995
Defined as positive or negative feelings that an individual holds about objects, persons or ideas.	Bargh, Chen, & Burrows, 1996
A relatively enduring organisation of beliefs, feelings and behavioural tendencies towards socially significant objects, groups, events or symbols, or; a general feeling or evaluation - positive or negative - about some person, object or issue.	Jones & Day, 1997

Table 2.7. continued.

Definition	Reference
Defined as a learned, global evaluation of an object (person, place or issue) that influences thought and action.	Perloff, 2003
A consumer's learned tendencies to evaluate brands in a consistently favourable or unfavourable way.	Assael, 2004
Strength of the consumer's belief with regard to an object of some sort, e.g. the company image or brand. It can be positive, negative or neutral. It has three components: beliefs; emotions; and behaviour.	Wright, 2006
A state of mind, mental view or disposition with regard to a fact or state.	Hillson & Murry-Webster, 2007
Relatively enduring overall evaluations of objects, products, services, issues or people.	Babin & Harris, 2009
Lasting general evaluation of something a consumer experiences, including how he feels about it and what he believes.	Lake, 2009
Consumer's evaluative response to an object or person; their feelings or affective reaction towards it.	Evans, Jamal, & Foxall, 2009
Enduring organization of motivational, emotional, perceptual, and cognitive processes, with respect to some aspect of the environment. It is a learned predisposition to respond in a consistently favourable or unfavourable manner, with respect to a given object.	Hawkins & Mothersbaugh, 2010
A lasting, general evaluation of people (including oneself), objects, advertisements, or issues.	Solomon, 2011
A learned construct that shows a person's tendency to respond to an object in a consistently favourable or unfavourable manner.	Blythe, 2013

Furthermore, attitude is an internal state of readiness that exists in the mind, organized through psychological tendencies, including experiences, and exerts a distinctive or dynamic influence upon an individual's response to all objects and situations, with which he or she is related (Du Plessis & Rousseau, 2003; Eagly & Chaiken, 1993; Zinkhan & Braunsberger, 2004). The attitude definition identities consist of four important distinct key concepts: evaluation function; psychological tendencies; responses to objects; and responses to situations. These concepts signify the processes that lead to attitude formation (Venter, 2006).

According to Albarracín and Kumkale (2003), the complexity of attitudes and their changing processes is influenced by both internal (e.g. perception, emotions, attitudes) and external (e.g. culture, demographics, social status) factors, that play a role in the formation and outcome of the attitude. These are the factors on which an individual bases his or her evaluative attitude judgement and decides whether to act on it or not. It should also be noted that each factor is independent of each other, though there is an interrelationship. For example, some consumers would rely more on internal than external factors to make an attitude evaluation or vice versa. This is one of the realities that complicate the measurement and changing processes of attitude (Crano & Prislin, 2006; Du Plessis & Rousseau, 1999).

By understanding the components of the attitude, it is possible to gain a better understanding of the consumer. Attitude has three main components, namely cognition, affect and conation. In line with Blythe (2008), cognition is the individual's awareness, knowledge, beliefs and images of the object of the attitude. It is the conscious, thinking part of attitude. Affect is the emotion, the feeling of like and dislike, which does not always have a basis in objective fact. Conation is about what consumers intend to do about the attitudinal object - whether to approach it; reject it; or buy it. It is not the actual behaviour, merely an intention (Blythe, 2008; Evans et al., 2009; Hawkins & Mothersbaugh, 2010). Figure 2.3 illustrates the factors that influence attitude formation.

2.10.2 Consumer attitudes towards new or unknown foods

Attitudes present a summary evaluation of an object. These evaluations can vary from positive to negative and they are experienced as affect. Typical evaluative dimensions are good or bad, pleasant or unpleasant and likeable or dislikeable (Ajzen, 2001). Positive attitudes are associated with approaching behaviour and negative attitudes are associated with avoidance behaviour (MacFie, 2007). In a study by Urala and Lähteenmäki (2004), consumers' attitudes

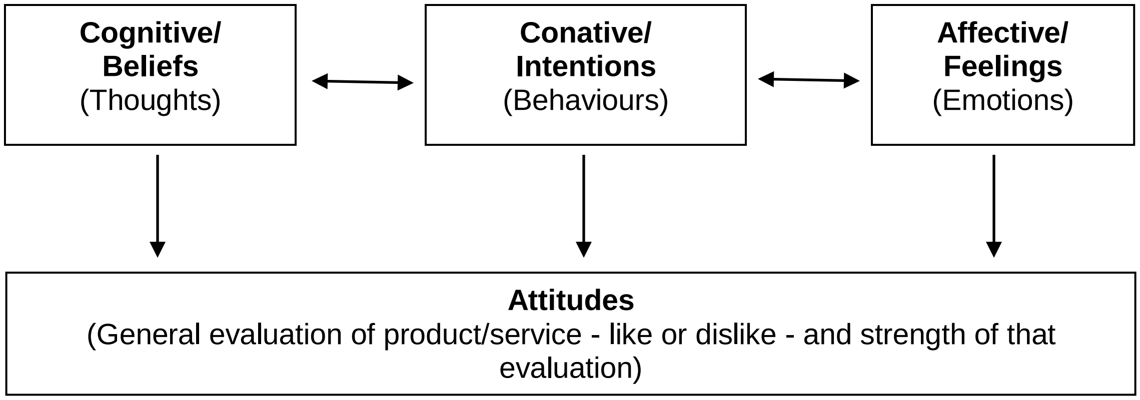


Fig. 2.3. Factors that influence attitude formation (Lake, 2009).

towards functional foods were determined. The study suggested that consumers have more positive attitudes towards physiology-related health benefits (e.g. osteoporosis) than towards benefits that are psychology related (e.g. stress). Attitudes toward new foods may not only have an impact on willingness to try new foods, but they may also influence the actual liking of a product (Urala & Lähteenmäki, 2004).

People's attitudes toward food are related to their other attitudes and beliefs. For example, the contradiction between nature and technology is important for a better understanding of the acceptance of food innovations. People tend to have confidence in natural food and the way it is produced, but they are suspicious of new foods and new food technologies (Huotilainen & Tuorila, 2005). Assessments of the naturalness of foods seem to be correlated with sensory appeal (Steptoe, Pollard, & Wardle, 1995). Natural food is associated with better appearance and better taste, compared to foods containing additives or artificial ingredients (MacFie, 2007).

Consumers, in general, are more positive about familiar foods than they are about unfamiliar foods (Raudenbush & Frank, 1999). Personality variables may also shape consumers' attitudes toward new foods. It has been demonstrated that some people have a stronger tendency to avoid new foods than other people, a phenomenon that has been labelled food neophobia. In a study by Coulthard and Blissett (2009), children were found to reject new fruits, vegetables and protein more readily than other categories of food. This has been explained as an adaptive evolutionary response, which prevents the ingestion of poisonous substances more commonly found in these classes of foods (Birch, 1999; Coulthard & Blissett, 2009). Both picky eating and neophobia have repeatedly been found to be associated with reduced consumption of fruit and vegetables. This finding has been in relation to both the amount and variety of fruit and vegetables consumed (Cooke, Carnell, & Wardle, 2006; Coulthard & Blissett, 2009).

Results of several studies suggest that food neophobia negatively influences consumers' willingness to try new foods. However, socio-demographic variables such as income, household size, education, age, gender and cultural background influence food-related behaviour. Nevertheless, attitudes may help to explain why some consumers buy new food products and why, at the same time, other consumers are hesitant to buy the very same food products. Cultural differences must, therefore, be taken into account when consumer attitudes towards new food technologies are examined (Urala & Lähteenmäki, 2004).

One aspect of individual differences that may be important in the determination of the refusal of new foods is the degree of an individual's sensory sensitivity. It is well established in literature that individuals vary according to the extent to which they perceive and respond to sensory information. The act of eating involves the processing of sensory information across a range of modalities: vision; touch; taste; smell (Coulthard & Blissett, 2009); and auditory.

In recent social psychological and cognitive models, two distinct processing modes have been identified. Based on these dual mode models, Slovic, Finucane, Peters and MacGregor (2004) drew a distinction between the analytical system and the experiential system. The analytical system uses probabilities or formal logic in making decisions. The experiential system, on the other hand, has a strong affective basis and is intuitive, fast and mostly automatic. Intuitive feelings are man's primary means of evaluating risks and this system helps to quickly decide whether something is good or bad. Slovic and colleagues assume that the affective reactions evoked by stimuli serve as cues for judgments. In accordance with this view, perceived benefits and perceived risks are shaped by the affect associated with a new or unknown food product. This phenomenon is known as the 'affect heuristic'. Slovic et al. (2004) used affect as it is employed in the concept of attitude (e.g. Ajzen, 2001), to mean overall degree of positivity or negativity toward the attitude object.

2.10.3 Predictors of attitudes

2.10.3.1 Health benefits

Novel or unknown foods (e.g. lucerne) may be more acceptable to the public if there are tangible benefits to the consumer (Frewer, Scholderer, & Lambert, 2003). Results suggested that acceptance of e.g., genetically modified (GM) products was largely determined by perceived risk and perceived benefit. Perceived benefits are much more important for the acceptance of GM products than perceived risks. Tangible benefits, such as products that are better for the environment or healthier, increased people's willingness to purchase them (MacFie, 2007). Two groups received information that differed in the presence or absence of a consumer benefit. The results showed that participants who were informed about a GM application with a consumer benefit, perceived lower personal risk, than those who were informed about an application without a consumer benefit. Recent studies suggest, however, that benefit alone does not guarantee acceptance. It should also be emphasised that consumers are not a homogeneous group. In other words, consumers differ in what they perceive as benefits. Therefore, tangible benefits may not result in higher acceptance for all food products; instead, it is contingent on consumer acceptability of specific applications. Attitudes towards new technologies are shaped by the perceived benefits associated with them (MacFie, 2007).

2.10.3.2 Food safety risks

Generally speaking, risk perception might be regarded as a form of attitude towards a specific object, such as a potential hazard. Risk may be conceptualised in terms of the risk to human health, the environment, animal health and future generations (MacFie, 2007; Miles & Frewer, 2001). Slovic (1987) reported that two dimensions were important when laypeople judge risks: the first aspect is the extent to which a hazard is 'dreaded' (severe, likely, uncontrollable, involuntary, catastrophic); and the second is how 'known' the hazard is (known to science, new, has delayed effects). In a study by MacFie (2007), people seemed to have a positive view of the risks to themselves from various hazards and also had a positive view of their own dietary behaviour, both in terms of intake of particular nutrients and of specific foods. It was suggested that perceptions of risks and benefits play an important role in shaping consumer attitudes towards new or unknown food.

Risk perception seems to be linked to social factors and individual differences have been identified in risk perceptions, related to environmental and food-related hazards. Some studies found that gender was a significant, but weak, predictor of the risk perceived (MacFie, 2007; Siegrist, 2000). Risk communication may have an effect when people do not hold strong convictions related to the new food product (Earle & Siegrist, 2006; MacFie, 2007). Slovic et al. (2004) suggested that the importance of the dreadfulness of a hazard for perceived risks can be viewed as evidence of 'risk as feelings'. Affect or attitudes seem to determine risk perception (MacFie, 2007).

Information conveyed by risk communication is, therefore, mediated by the attitudes people hold. Scholderer and Frewer (2003) examined the effects of various information strategies on consumer attitude change towards GM foods. Results indicated that the information strategies used by the researchers decreased consumers' acceptance of GM foods, compared to the control group. The authors concluded that the information material was more likely to activate pre-existing attitudes than the no-additional-information condition in the control group. The activation of the pre-existing attitudes resulted in an increased consistency of the beliefs and choices expressed by the participants. People's attitude toward GM foods seems to be so strong that new information is overridden. Informing the public about new food products may often fail to increase acceptance, unless other factors (such as personal or societal benefits and the values placed on these) are also addressed (MacFie, 2007). Attitudes toward GM technology are influenced by more general environmental attitudes. The attitude of favouring the protection of nature, because of its intrinsic value, had a negative impact on the acceptance of GM technology. Valuing nature because of its usefulness and benefits to humans, however, had a positive influence on the acceptance of GM technology. In a similar study, general attitudes or world views had an important influence on the perception of GM technology (MacFie, 2007; Siegrist, 1999).

The concept of attitudes is a psychological approach toward a better understanding of the acceptance or non-acceptance of novel or unknown food (e.g. lucerne). However, a psychological view may be too narrow. Attitudes toward a new food technology will be influenced, not only by the innovation itself, but also by the surrounding social, economic and political environments. Various dynamic social processes may generate public concern about hazards that are judged as low risks by experts, to the neglect of hazards that they judge as high risks (Kasperson, Kasperson, Pidgeon, & Slovic, 2003).

2.10.3.3 Sensory qualities

Aikman and Crites (2007) suggested that food attitudes are comprised of five distinct informational bases: positive effect (e.g. calm, comforted); negative effect (e.g. guilty, ashamed); abstract cognitive qualities (e.g. healthy, natural); general sensory qualities (e.g. taste, smell); and specific sensory qualities (e.g. salty, greasy).

2.10.3.4 Synonyms

The name lucerne is commonly used in all European countries east of Spain, and also in SA, Australia and New Zealand (NLO, 2010). Since North and South America now produce a large part of the world's output, the word "alfalfa" has been slowly entering into other languages besides English. Names, derived from the Germanic language (e.g. English), Romance language (e.g. French or Italian), or non-Indo-European language (e.g. Chinese or Swahili), can help create a different attitude towards a food product. Exploring other languages when naming, is an obvious approach when the target consumer market includes many non-English speakers. However, non-English names can also be appealing to native English speakers, especially when they are familiar foreign words (Catchword Branding, 2013).

2.10.4 Measuring attitudes

Explicit and implicit attitude methods have been used to measure attitudes toward food (Gawronski & Bodenhausen, 2006; Greenwald & Banaji, 1995; Greenwald, McGhee, & Schwartz, 1998). As observed by Gawronski and Bodenhausen (2006), explicit attitudes are usually equated with deliberative, self-reported evaluations; the latter are typically inferred from people's performance on response latency measures, such as the Implicit Association Test (IAT; Greenwald et al., 1998) or sequential priming tasks (Fazio, Jackson, Dunton, & Williams, 1995; Wittenbrink, Judd, & Park, 2001). Roininen, Lähteenmäki and Tuorila (1999) described a scale that measures the importance of health and taste characteristics. Other scales measured attitudes toward new food and food technology (Huotilainen & Tuorila, 2005). Most of these scales are not pure attitudinal measurements and include mixtures of attitudinal items, behavioural intentions and beliefs (MacFie, 2007).

The Likert Scale presents a set of attitude statements that is easy for researchers to prepare and to interpret, and simple for consumers to answer (Schiffman & Kanuk, 2010). Subjects are asked to express agreement or disagreement on a five-point scale, where each degree of agreement is given a numerical value from one to five. Thus, a total numerical value can be calculated from all the responses. This method allows respondents to give answers that relate to positive and negative attitude strengths. The answers can then be coded and used in a quantitative manner. The disadvantage of using the Likert Scale is that it fails to take into account mental complexities, and the relative strengths and weaknesses of the beliefs and feelings that make up an individual's attitudes toward product issues. A more useful approach is to make use of a multi-attribute model (Wright, 2006), such as the Theory of Planned Behaviour (TPB) (Ajzen, 1991), which evolved from the Theory of Reasoned Action (Fishbein, 1967).

2.10.5 Theory of Planned Behaviour (TPB)

The TPB (Ajzen, 1991) proposes a model about how human action is guided. It predicts the occurrence of a specific behaviour, provided that the behaviour is intentional. The model is depicted in Figure 2.4 and represents the three variables, namely attitudes, subjective norms and perceived behavioural control, which will predict the intention to perform behaviour. Intentions are the precursors of behaviour. Attitude toward the behaviour is a person's overall evaluation of the behaviour and is assumed to have two components which work together: beliefs about consequences of the behaviour (behavioural beliefs); and the corresponding positive or negative judgements about each of these features of the behaviour (outcome evaluations).

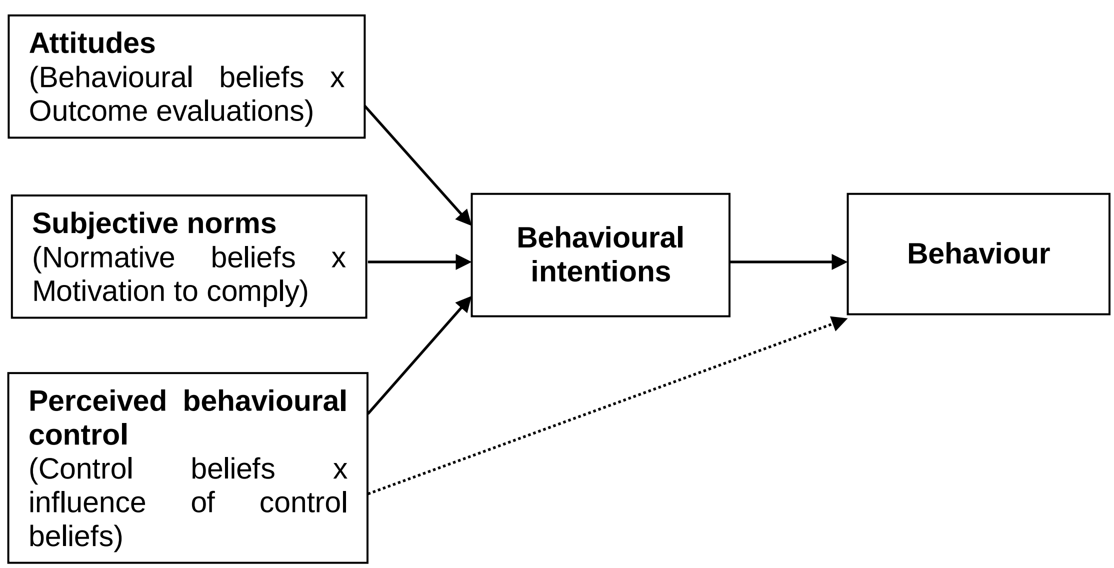


Fig. 2.4. The Theory of Planned Behaviour model (Ajzen, 1991).

2.11 Conclusions

Lucerne is generally known as a roughage source for livestock. It is important for SA consumers and researchers to become aware of the following: (1) the nutritional potential for consumption purposes in the future, in order for the lucerne industry to diversify, to contribute to the unsatisfied diet status of SA consumers and to target malnutrition; (2) there are more interest in the use of lucerne as consumers' food and production need to be continuously adapted to meet changing demands of society; (3) integrating lucerne into human diets can be promoted as the most practical and sustainable way to achieve food sustainability in SA; and (4) the exorbitant cost of protein from animal sources will continuously lead to a growing interest in industrial application of protein of plant origin. This could challenge the increasing demand for protein in the food and non-food market. Fierce competition in the food market forces companies to differentiate and add value to their products through these underutilised food ingredients and flavours. Research efforts, on the evaluation of the potential of lucerne for industrial applications in the food field, will hold some challenges, but could contribute to food security, human nutrition, breeding programmes and convenient sources of income for effective and sustainable development in SA.

Three components, namely nutritional and microbial composition of lucerne, sensory analysis and consumer attitudes, will be addressed in this study to investigate the full development of lucerne's potential as a sustainable food source in SA.

CHAPTER 3

THE CHEMICAL AND MICROBIAL COMPOSITION OF LUCERNE (*Medicago sativa* L.)

ABSTRACT

The chemical and microbial content of three lucerne (*Medicago sativa* L.) cultivars ('SA Standard', 'WL711' and 'WL525') and one spinach beet (*Beta vulgaris* var. *cicla* L.) cultivar (as control) were investigated in order to determine the nutritional benefits of lucerne, as well as to determine the cultivar to use for future research. Degrees Brix and amino acids were determined for raw cultivars. Macro- and micro-minerals, protein, dry matter, moisture, ash, fat, fibre, carbohydrate and energy contents; average mineral cooking losses; microbial counts and shelf-life were determined for both raw and cooked cultivars. Analysis of variance determined differences between cultivars. The Tukey-Kramer multiple comparison test ($\alpha=0.05$) was used to determine differences between cultivar means. The Brix content of the lucerne cultivars was significantly ($p<0.01$) higher than spinach beet (SB). Raw lucerne cultivars had significantly ($p<0.01$; $p<0.001$) higher Ca and S contents than raw SB. The K, S and Mn content for the raw lucerne cultivars, were significantly ($p<0.001$) higher than the cooked cultivars. The protein content of the raw lucerne cultivars were significantly ($p<0.001$) higher than the raw SB. Cooked 'SA Standard' and 'WL525' had significantly ($p<0.001$) higher protein contents than cooked SB. Both raw and cooked 'SA Standard' had a significantly ($p<0.001$) higher carbohydrate content than both raw and cooked 'WL525', 'WL711' and SB. Raw 'SA Standard' had a significantly ($p<0.001$) higher energy content than raw 'WL525', 'WL711' and SB. Cooked 'SA Standard' had a significantly ($p<0.001$) higher energy content than cooked 'WL525' and SB. The lucerne cultivars contained higher values of essential and non-essential amino acids than SB. The total bacterial count, coliform count, lactic acid bacteria count and mould count conformed to standards while cooking significantly ($p<0.001$) reduced the bacterial load. No *E. coli* or coliforms were detected in any of the raw and cooked cultivars and SB. For the microbial shelf-life study, it was concluded that the cooked lucerne and SB samples were of satisfactory microbiological quality until day seven. Based on these findings, lucerne cultivar 'SA Standard' was proposed for further studies.

3.1 Introduction

Lucerne is the most widely grown forage crop in the world and it is one of the plants producing the most protein per hectare (EFSA, 2009). Lucerne contains high nutrient levels, high digestibility and unique proportions of structural to non-structural components (Yari et al., 2012). The nutritional value of lucerne hay in South Africa (SA) has been researched by Scholtz et al. (2009), mainly for animal grazing purposes. However, little information is available about the nutritional value of fresh lucerne leaves, purposive for human consumption.

Soil is the main source of nutrients for crops and provides support for plant growth in various ways. Knowledge about soil health is critical to sustain crop productivity (Motsara & Roy, 2008). Before considering the cultivation of lucerne, it is necessary to conduct a comprehensive investigation of the soil, to ensure that it would be possible to grow lucerne economically and to ensure an increased availability of minerals. This research study focused on extractable calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P) and soil pH, because these properties mostly account for soil infertility and affect plant nutrition (PBPM, 2011).

Epiphytic microflora are naturally present on forage crops such as lucerne, and some are responsible for silage fermentation (Lin et al., 1992). However, scant information is available on the microorganisms present on fresh lucerne leaves. Pre-harvest, harvest and post-harvest factors can affect green leafy vegetables' microbial status. Adjrah et al. (2011) and Halablab et al. (2011) stated that pre-harvest factors such as water, growth conditions, environment, soil, dust, animals (including birds and insects) and animal faeces, as well as the equipment and transportation that are used during harvesting will influence the number of microorganisms. All leafy vegetables are subject to primary preparation and are therefore exposed to food workers, multiple contact surfaces, water, processing aids and the process environment (FAO, 2008). Processing such as cooking will have an effect on the microbial status of the lucerne product, which will determine the microbiological shelf-life of the product under refrigerated conditions (Marth, 1998).

Therefore, the objective of this chapter was to investigate the chemical (degrees Brix, macro- and micro-minerals, protein, amino acids, dry matter [DM], moisture, ash, fat, fibre, carbohydrates and energy contents) composition and microbial content, and shelf-life of three lucerne cultivars and compare it to SB (a familiar leafy vegetable as control), to determine which lucerne cultivar to use for future research purposes in food product development. Spinach beet was chosen, as it is a well-known, green, leafy vegetable consumed in SA. According to Kerr (2014), baby spinach (*Spinacea oleracea* L.) is more difficult to grow than spinach beet, and less productive, because of its inability to adapt to extreme temperatures.

3.2 Materials and methods

3.2.1 Evaluation of soil

Before the establishment of lucerne cultivars, the soil of the trial site was analysed by NviroTek Laboratories Pty Ltd, Brits, to determine if any deficiencies in soil pH, P, K, Ca and Mg levels needed to be corrected (PBPM, 2011). Soil pH was measured in 1 N potassium chloride (KCl) extract at a 1:1 soil to solution ratio, using a Beckman Expandomatic pH meter. The soil available P was determined according to Bray No. 1 methods (Bray & Kurtz, 1945), using an auto analyser (IITA, 1982). Potassium, Ca and Mg, present in the soil sample, were extracted with neutral 1 M ammonium acetate, by shaking the soil-extract mixture (Motsara & Roy, 2008). The extraction was then measured by an inductively coupled plasma-mass spectrometry emission spectroscopy (ICP-MS) instrument (Ziadi & Sen Tran, 2007).

3.2.2 Establishment of cultivars

Three lucerne cultivars, 'SA Standard' ('SAS') (Code: 2010029), 'WL525' (Code: 1020249) and 'WL711' (Code: 1017152) and spinach beet (SB; *Beta vulgaris* var. *cicla* L.; control) were cultivated in Potchefstroom in the North-West Province, during January 2012, in the morning at a temperature of 25 °C, humidity of 64% and wind speed of 12 km/h. The pastures were irrigated weekly during the summer mornings. To ensure good irrigation practice, the criteria of the PBPM (2011) was followed. Lucerne requires 0.65 – 0.80 mm of water to produce 10 kg of lucerne. Potchefstroom is situated in the summer rainfall region of SA, with a long term average annual rainfall of 613 mm, occurring mainly between September and April. The high evaporation rates of the area imply a water deficit during the whole year (Aucamp, 2000).

Further information of each cultivar is provided in Chapter 2 (Table 2.2). These three cultivars were of high quality, certified seed and were purchased from K2 Agri Klein Karoo Seed Marketing Pty Ltd., in Potchefstroom in the North-West Province. The seeds were already inoculated by K2 Agri with the appropriate *Rhizobium* nodule bacteria to convert atmospheric nitrogen to a form available to the plant (PBPM, 2011). These cultivars were chosen, as they provide better germination rates and establishment, better DM production, better aphid and disease resistance, and are weed-free (K2 Agri, 2011).

To ensure good establishment, the criteria of the PBPM (2011) was followed. A well-drained, sandy clay loam (Mbatani, 2000) paddock was chosen and sprayed out with a rake to eliminate all weeds (including grasses). The seedbed was made even with no compaction layers. For lucerne, seed was sown at 12 – 18 kg/ha and planted no deeper than 25 mm (PBPM, 2011). For SB, seed was sown at 7 – 9 kg/ha and planted no deeper than 20 mm (SADAFF, 2013).

3.2.3 Sampling procedure

All the samples were harvested in the morning, when the dew or moisture was high, to limit leaf loss (Dickinson et al., 2010). The lucerne leaves were sampled during the mid-vegetative (stem length 15 – 30 cm, no buds, flowers or seed pods) to late vegetative stage (stem length greater than 30 cm, no buds, flowers or seed pods) (see annexure 2). Lucerne leaves contain 70% of the protein and 90% of the vitamins and minerals, found in the lucerne crown. Therefore, lucerne quality is highest when harvesting takes place at an early stage of development, when the leaves have a high percentage of the total DM yield (PBPM, 2011).

Proper handling of the lucerne samples was ensured by hand picking clean samples (the upper 150 mm or 1/3 of the plant) and placing it in clean brown paper bags. Samples should not be placed in plastic bags or sealed containers as this promotes mould growth (PBPM, 2011). Samples damaged by insects or climatic conditions were avoided and plant tissues were taken from the same relative position of the plant.

The SB leaves were harvested when the leaves were 10 – 12.5 cm long, by twisting the outer leaves sideways. Only leaves that were free of insect injury, worms, moulds, decay or other serious injury that affects its appearance, were picked. As with the lucerne samples, SB samples were placed in clean brown paper bags (SADAFF, 2013). All samples were cooled at 4 °C and evaluated within 24 – 48 hours. All chemical and microbial analyses of the cultivars and SB were performed in triplicate.

3.2.4 Preparation of cooked samples

Lucerne and SB leaves were cooked according to Mepba, Eboh and Banigo (2007). The leaves were cooked in filtered water at 96 °C for 10 min, and then cooled and stored at 4 °C, in order to prevent deterioration and to perform further chemical and shelf-life microbial analysis. One percent of sodium chloride (NaCl) was added to the cooking water, as Kimura and Itokawa (1990) found that a 1% addition of NaCl reduced the loss of mineral constituents in cooked spinach.

3.2.5 Degrees Brix

The degrees Brix was analysed by NviroTek Laboratories Pty Ltd, Brits, according to the AOAC 932.12 method (AOAC, 1990). Juice was extracted from the cultivar leaves by crushing the leaves with a garlic press. A drop of juice was then placed on the receptacle of a PAL Digital Pocket Refractometer (Atago, Japan) to determine Brix percentage.

3.2.6 Macro- and micro-minerals of lucerne

The macro- and micro-minerals were analysed by NviroTek Laboratories Pty Ltd, Brits, according to the wet digestion method for plant analysis (FAO, 2008). The samples were brought into solution through digestion with acids that dissolve the solid plant parts and convert the plant nutrients to a liquid for analysis (known as wet digestion). A mixture of nitric acid (HNO_3), sulphuric acid (H_2SO_4) and perchloric acid ($HClO_4$), in the ratio of 9:4:1, was used for tri-acid digestion. For di-acid digestion, two acids, HNO_3 and $HClO_4$ (9:4), were used. While di-acid digestion was used for determination of Ca, Mg, sulphur (S), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu), tri-acid digestion was used for P and K analysis (Motsara & Roy, 2008).

For mineral determination, 1 g of plant sample was ground with an electric stainless steel mill and placed in a 100 ml volumetric flask. Ten millilitres of acid mixture was added and the contents were mixed by swirling. The flask was placed on a hotplate in a fume hood and heated, starting at 80 – 90 °C, where after the temperature was raised to ≈ 150 – 200 °C. Heating continued past the production of red nitrogen dioxide (NO_2) fumes ceased, until the volume was reduced to 3 – 4 ml and became colourless (Motsara & Roy, 2008). After cooling the contents, the volume was made up to 50 ml (Ahmad et al., 2013) with distilled water and filtered through Whatman No. 1 filter paper. The filtrate was then measured by an inductively coupled plasma-mass spectrometry emission spectroscopy (ICP-MS) instrument (Ziadi & Sen Tran, 2007).

3.2.7 Average mineral cooking losses

The cooking losses of the macro- and micro-minerals were calculated on a wet weight basis for each replicate. The replicate results were averaged for the sample being analysed. The average value obtained from subtracting the specific mineral concentration, before and after cooking, was considered a loss if positive, or an apparent gain if marked with a positive symbol (Kawashima & Valente Soares, 2003).

$$\% \text{ Cooking loss} = \frac{(\text{MC of raw sample} - \text{MC of cooked sample})}{\text{MC of raw sample}} \times 100$$

Where, MC = Mineral concentration

3.2.8 Sample preparation for dry matter, moisture, ash, fat, fibre, carbohydrates, energy, protein and amino acids contents

All samples were freeze-dried and milled to obtain a homogeneous sample. The total DM, moisture, ash, protein, amino acids (AA), fat, fibre, carbohydrates and energy content were determined in triplicate by the ARC-Irene Analytical Service, Pretoria (Facility Accreditation Number: T0063), which is accredited in accordance with the recognised International Standard: ISO/IEC:17025:2005. All the results were calculated, using the freeze-dried values and the final results were expressed on a wet weight basis.

3.2.8.1Dry Matter

Analytical Standard Method (ASM) 013 was used to determine the percentage DM (AOAC, 2000). This method was chosen, as it is suitable for the determination of DM in animal feed and animal faeces, as well as plant tissue samples and food products. The DM content is the residue expressed in % by weight, which remains after the drying process. The moisture from a sample is driven off by use of heat (oven drying at 105 °C for 16 h). Weight loss is used to calculate DM content, by the following formula:

$$\% \text{ Total DM} = \frac{W_6 - W_4}{W_5 - W_4} \times 100$$

Where, W_4 = tare weight of dish in g

W_5 = initial weight of sample and dish in g

W_6 = dry weight of sample and dish in g

3.2.8.2 Moisture

Moisture was determined by the ASM 013 method (AOAC, 2000). Moisture was estimated by the gravimetric method where the loss in weight at a constant temperature was measured. A 2.0 g sample, in a pre-weighed glass weighing bottle, was heated in a temperature-controlled oven for about 16 h at 105 °C. The sample was cooled in a desiccator and weighed. The following formula was used to calculate the % moisture:

$$\text{Moisture \% by weight} = \frac{(B - C) \times 100}{B - A}$$

Where, A = weight in grams of the empty sample bottle

B = weight in grams of the bottle plus material before drying

C = weight in grams of the bottle plus material after drying

3.2.8.3 Ash

The percentage ash was determined according to ASM 048 (AOAC, 2000). The total ash is the inorganic matter of a sample. The organic matter of a sample is removed by heating at 550 °C overnight. The remaining residue is inorganic matter (ash). To calculate the % ash, the following formula was used:

$$\text{\% Ash (DM basis)} = \frac{(W_3 - W_1) \times 100}{(W_2 - W_1) \times DM/100}$$

Where, W_1 = tare weight of crucible in g

W_2 = weight of crucible and sample in g

W_3 = weight of crucible and ash in g

3.2.8.4 Fat analysis

The fat content was determined according to ASM 044 by means of the Soxtec method (AOAC, 2000). Most of the fat is soluble in petroleum ether (PE) and dissolves in the ether at boiling point. The ether is evaporated at 105 °C and the fat remains in the beaker. Weight gain is used to calculate fat content. Bounded fat is not soluble in PE. The following formula was used to calculate the % fat:

$$\text{\% Crude fat (DM basis)} = \frac{(W_3 - W_2) \times 100}{W_1 \times DM/100}$$

Where, W_1 = initial sample weight in g

W_2 = tare weight of beaker in g

W_3 = weight of beaker and fat residue in g

3.2.8.5 Fibre analysis

Fibre content was determined according to ASM 059 (AOAC, 2000). It is the determination of crude fibre by means of the fritted glass crucible method. Crude fibre is loss on ignition of dried residue remaining after digestion of the sample with 1.25% H_2SO_4 and 1.25% NaOH solutions under specific conditions. In this method the sample was exposed to the minimum vacuum needed to regulate filtration, and heating of the sample solutions prevented gelling or precipitation of possible saturated solutions. To calculate the % fibre, the following formula was used:

$$\% \text{ Crude fibre} = \frac{\text{Loss in weight on ignition} \times 100}{\text{Weight sample}}$$

3.2.8.6 Carbohydrates

The carbohydrate content was analysed using ASM 075 (AOAC, 2000). Moisture, crude protein, ash and fat were determined individually, summed and subtracted from the total weight of the sample. This is referred to as *total carbohydrate by difference* and is calculated by the following formula:

% Total carbohydrates = 100 - (weight in g [protein + fat + water + ash + alcohol] in 100 g of food)

3.2.8.7 Energy

The energy content (dry material) was determined according to ASM 074 (AOAC, 2000). Metabolisable energy (ME) can be calculated by using energy conversion factors for protein, fat and carbohydrates content. To calculate the ME, the following formula was used:

$$ME = (GE_p - 7.9/6.25)D_p + GE_fD_f + GE_{cho}D_{cho}$$

Where, GE_p, GE_f and GE_{cho} = the gross energy of protein, fat and carbohydrate, respectively

D_p, D_f and D_{cho} = the digestibility coefficient of protein, fat and carbohydrate, respectively

3.2.8.8 Protein analysis

The Kjeldahl method (ASM 056; AOAC, 2000), which measures total organic nitrogen (N), was used to determine the protein content of raw and cooked leaves. The organic matter is digested with hot concentrated H_2SO_4. A catalyst mixture is added to the acid, to raise the boiling point. All N is converted to ammonia (NH_3), which is measured by titration. A conversion factor of 6.25 was used in the calculation of the protein content from the total nitrogen (N). The following formula was used to determine the % N:

$$\% N = \frac{[(\text{ml standard acid x normality of acid}) - (\text{ml blank x normality of base})] - (\text{ml standard base x normality of base}) \times 1.4007}{\text{Weight of sample in g}}$$

Where, ml blank = ml of base needed to back titrate a reagent blank if standard acid is the receiving solution

% Protein = % N x 6.25

3.2.8.9 Amino acids

The AA content was determined according to ASM 021 (AOAC, 2000). Seventeen AA were analysed by High Performance Liquid Chromatography (HPLC) (Einarsson, Josefsson, & Lagerkvist, 1983), with a limit of quantification of 0.006 g/100 g and a limit of detection of 0.004 g/100 g. The replicate results of the essential and non-essential AA were averaged to illustrate the difference between essential and non-essential AA of the tested cultivars.

The amino acid score (AAS) was then calculated. Firstly, the limiting amino acid (LAA) was identified by using a reference AA scoring pattern, in which AA requirements were expressed as mg/g protein. Alternatively, it can also be expressed as percentages needed in a theoretically ideal dietary protein, to meet the requirements of all indispensable AA, when the amount consumed meets the N requirement. Amino acid scores were then calculated, by expressing the amount of each AA in the dietary protein, as a percentage of the amount in the scoring pattern. Values >100% were considered to be 100; then, from among those >100, the LAA was identified as the one having the lowest score (Harper & Yoshimura, 1993). The provisional AA scoring pattern of the FAO (1981) was used for calculating the AAS of the lucerne cultivars:

$$AAS = \frac{\text{Test AA} \times 100}{\text{Reference AA}}$$

3.2.9 Microbial analysis

The microbial analysis of the samples was performed by the Food Microbiology Laboratory in the Department of Microbial, Biochemical and Food Biotechnology, University of the Free State (UFS), Bloemfontein. For the determination of the microbial load, the following evaluations were performed in triplicate on the raw and cooked cultivars and SB. Ten grams of the leaves were weighed into a WhirlPak™ bag, 90 ml of 0.1 M phosphate buffer was added and homogenized (Lab Blender 400, ART Medical Equipment) for 1 min. Further serial dilutions were prepared to 10^{-10}, and 1 ml of the appropriate dilutions was pour-plated, using standard plate count agar (SPCA; Oxoid CM0463) for the determination of the total bacterial counts (TBC). Plates were incubated at 32 °C for 48 h. After incubation, the colonies were enumerated by means of a colony counter (Harrigan, 1998).

For determination of the coliform count and *E. coli*, lactic acid bacteria (LAB) count and yeast and mould counts, serial dilutions were prepared to 10^{-5}. Violet red bile agar with MUG (VRB+MUG; Oxoid CM0978) was used for enumeration of coliforms and *E. coli,* and incubated at 37 °C for 24 h. The presence of *E. coli* was confirmed by fluorescence of the colonies under ultraviolet light. For LAB, dilutions were pour-plated on MRS agar (Oxoid CM0359). The plates were incubated at 32 °C for 48 – 72 h. The pour-plate method was also used for determination of yeast and mould counts, using rose bengal chloramphenicol agar (RBC; Oxoid CM0549) and plates were incubated at 25 °C for 4 – 5 d (Harrigan, 1998).

3.2.10 Microbial shelf-life

The shelf-life analysis of the samples was performed by the Food Microbiology Laboratory in the Department of Microbial, Biochemical and Food Biotechnology, UFS, Bloemfontein. The cooked samples were placed at 4 ^{o}C and the same microbial analysis performed in paragraph 3.2.9, were used to evaluate the samples in triplicate on days 0, 3, 5, 7, 10 and 12.

3.2.11 Statistical analysis

Data was entered in Microsoft Excel 2010 spread sheets and statistical analyses were performed on XLSTAT 2007 (http://www.xlstat.com). Differences between cultivars for chemical and microbial attributes were determined by using analysis of variance (ANOVA) for balanced data. The Tukey-Kramer multiple comparison test (α=0.05) was used to determine differences between cultivar means (NCSS, 2007).

3.3 Results and discussion

3.3.1 Evaluation of soil

The pH of the soil sample was 6.14. According to the PBPM (2011), the optimum soil pH for lucerne is 6.5 – 6.8. If the soil pH is between 4.8 and 5.5, lucerne production may be reduced, as lucerne is very sensitive to acid soils (Dickinson et al., 2010). Below pH 4.8, satisfactory establishment will be difficult and lucerne growth will be markedly reduced. Although lucerne is reasonably resistant to brack conditions, production is adversely affected if the pH (KCl) rises above 6.5 (Dickinson et al., 2010). Tongel and Ayan (2010) stated that lucerne can produce over 20 ton DM/ha on well drained, high fertile soils, with a pH > 5.8. The pH content of the soil in this study was, therefore, satisfactory. Phosphorus and K are made more available at neutral pH levels, while Mg, Fe and aluminium (Al) become more toxic to the lucerne plant at low soil pH (PBPM, 2011). The benefits of maintaining pH levels include (PBPM, 2011): i) increased stand establishment, early growth and crop vigour; and ii) increased N fixation.

The mineral composition of the soil is shown in Table 3.1. The P content of the soil sample was 61 mg/kg. According to Dickinson et al. (2010), lucerne requires a soil P of 25 mg/kg under dryland conditions. At this level further P applications will probably not be necessary. Under irrigated conditions, no P is required during establishment if the soil has a P content of 40 mg/kg.

Table 3.1

The mineral composition of the soil used for lucerne cultivar establishment in this study.

Mineral	Test results (mg/kg)	Standards (mg/kg) (Dickinson et al., 2010)
Phosphorus (P)	61	> 25
Potassium (K)	282	> 120
Magnesium (Mg)	636	> 60
Calcium (Ca)	2249	> 2000

The K content of the soil sample was 282 mg/kg. Above 120 mg/kg, little reaction to K applications can be expected. Below 50 mg/kg, growth vigour can decline and symptoms of deficiency can occur. For Mg, the content of the soil sample was 636 mg/kg. Symptoms of

Mg deficiency can occur at contents below 60 mg/kg. The Ca content of the soil sample was 2249 mg/kg. Lucerne thrives in soils with Ca levels above 2 000 mg/kg (Dickinson et al., 2010). It was concluded that the mineral composition of the soil in this study with regard to P, K, Mg and Ca was satisfactory for lucerne establishment.

3.3.2 Degrees Brix

Results in Table 3.2 indicate that the Brix content of the raw lucerne cultivars (16.13% / 18.90% / 16.00%) were significantly ($p<0.01$) higher than the raw SB (4.77%). According to the Reams' Brix quality chart (Harrill, 1998), the refractive index of lucerne juice, calibrated in % sucrose or °Brix, is classified as: poor = 4; average = 8; good = 16; and excellent = 22. In this study, 'SAS' (16.13%) and 'WL711' (16.00%) indicated good Brix values, while cultivar 'WL525' (18.90%) indicated a good to excellent Brix value. Harrill (1998) stated that as a general rule of thumb, Brix readings above 12 confer reasonable plant pest immunity.

Spinach beet had a mean Brix value of 4.77%. This is in agreement with Kleinhenz and Bumgarner (2012), who indicated an average Brix value of 4.6% for SB. However, Mwazi, Amoonga and Mubiana (2010) indicated a Brix value of 2% for SB. These results can be explained by the total sugar content in fruits and vegetables, as a function of genetic, nutritional and environmental factors (Nookaraju et al., 2010; Türkmen & Ekşi, 2011). Although variety affects taste, this will not change the fact that a high Brix food product of any produce is better tasting than a low Brix food product of the same produce (for instance, some consumers prefer tart apples and some like sweet varieties). Therefore, the high Brix values of the lucerne cultivars are not necessarily better tasting, than the low Brix value of the SB cultivar (Harrill, 1998).

3.3.3 Macro- and micro-minerals

When comparing the macro- and micro-mineral content for both the raw and cooked lucerne cultivars with the raw and cooked SB, no significant differences were found in the P and Cu content (Table 3.2). In a study by Kołota, Adamczewska-Sowińska and Czerniak (2011) the mean P content for five SB cultivars was 0.40%, which is in agreement with the results in Table 3.2. Bozokalfa, Yağmur, Aşçioğul and Eşiyok (2011) studied the diversity in mineral composition of SB and found the Cu content to be 10.42 – 13.66 µg/g, which is in

Table 3.2

The Brix (%), macro- (%) and micro-mineral (µg/g) composition of the raw and cooked lucerne cultivars and the spinach beet.

	Spinach beet Raw	Spinach beet Cooked	'SA Standard' Raw	'SA Standard' Cooked	'WL525' Raw	'WL525' Cooked	'WL711' Raw	'WL711' Cooked	Sign. Level
Brix (%)	4.77 ± 2.89^{a}	ND	16.13 ± 3.33^{b}	ND	18.90 ± 1.68^{b}	ND	16.00 ± 2.78^{b}	ND	**
P (%)	0.47 ± 0.06	0.26 ± 0.03	0.36 ± 0.01	0.38 ± 0.11	0.35 ± 0.02	0.41 ± 0.12	0.38 ± 0.05	0.43 ± 0.17	NS
K (%)	3.48 ± 0.64^{b}	1.14 ± 0.15^{a}	2.78 ± 0.67^{b}	0.53 ± 0.07^{a}	2.89 ± 0.61^{b}	0.56 ± 0.13^{a}	3.29 ± 0.48^{b}	0.49 ± 0.06^{a}	***
Ca (%)	1.40 ± 0.25^{a}	2.06 ± 0.16^{ab}	2.59 ± 0.48^{b}	2.15 ± 0.22^{ab}	2.43 ± 0.27^{b}	1.96 ± 0.20^{ab}	2.38 ± 0.42^{b}	1.96 ± 0.15^{ab}	**
S (%)	0.46 ± 0.04^{a}	0.46 ± 0.02^{a}	0.69 ± 0.06^{b}	0.46 ± 0.02^{a}	0.65 ± 0.10^{b}	0.45 ± 0.02^{a}	0.62 ± 0.05^{b}	0.45 ± 0.01^{a}	***
Mg (%)	1.52 ± 0.41^{b}	0.66 ± 0.13^{a}	0.51 ± 0.06^{a}	0.33 ± 0.02^{a}	0.48 ± 0.06^{a}	0.32 ± 0.04^{a}	0.43 ± 0.06^{a}	0.32 ± 0.05^{a}	***
Zn (µg/g)	37.76 ± 14.88^{ab}	35.00 ± 1.73^{ab}	40.87 ± 4.27^{ab}	25.67 ± 3.06^{a}	43.29 ± 6.40^{ab}	44.67 ± 2.31^{b}	45.72 ± 3.75^{b}	42.00 ± 0.01^{ab}	*
Fe (µg/g)	310.37 ± 72.99^{ab}	343.33 ± 23.59^{b}	215.00 ± 53.70^{ab}	231.33 ± 91.13^{ab}	271.67 ± 47.01^{ab}	176.33 ± 21.55^{a}	270.67 ± 38.37^{ab}	323.00 ± 7.00^{b}	*
Mn (µg/g)	113.33 ± 3.06^{e}	47.67 ± 11.02^{a}	68.33 ± 4.93^{bcd}	47.67 ± 6.03^{a}	73.00 ± 10.44^{cd}	49.33 ± 0.58^{ab}	84.00 ± 8.66^{d}	60.33 ± 0.58^{abc}	***
Na (µg/g)	400.45 ± 52.57^{a}	527.08 ± 23.42^{ab}	464.33 ± 65.62^{ab}	1047.00 ± 193.27^{c}	530.00 ± 17.52^{ab}	661.33 ± 34.02^{b}	475.33 ± 37.98^{ab}	661.00 ± 77.93^{b}	***
Cu (µg/g)	12.46 ± 0.80	13.00 ± 2.65	13.48 ± 2.17	13.33 ± 0.58	12.73 ± 0.64	14.33 ± 2.31	12.07 ± 1.01	16.00 ± 3.61	NS

Means with different superscripts in the same row differ significantly.

P = phosphorus; K = potassium; Ca = calcium; S = sulphur; Mg = magnesium; Zn = zinc; Fe = iron; Mn = manganese; Na = sodium; Cu = copper.

NS = Not significant; * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$; ND = Not determined agreement with the results in Table 3.2. There were no significant differences in the K, Zn, Fe and Na contents of the raw lucerne cultivars and the raw SB. The Ca content for the raw lucerne cultivars (2.59% / 2.43% / 2.38%) was significantly ($p<0.01$) higher than the raw SB (1.40%). The PBPM (2011) recorded the normal range for Ca as 0.51 – 3.00% for raw lucerne. Raw 'SAS' (0.69%), 'WL525' (0.65%) and 'WL711' (0.62%) had a significantly ($p<0.001$) higher S content than raw SB (0.46%). The PBPM (2011) recorded the normal range for S as 0.26 – 0.50% in lucerne cultivars. The S content for the lucerne cultivars was, therefore, above the normal range. The Mg and Mn content for raw SB (1.52% / 113.33 µg/g) was significantly ($p<0.001$) higher than the three raw lucerne cultivars (0.51% / 0.48% / 0.43%; 68.33 µg/g / 73.00 µg/g / 84.00 µg/g) (Table 3.2).

No significant differences were found for the K, Ca, S, Mg and Mn contents between the cooked lucerne cultivars and the cooked SB (Table 3.2). The Zn content for the cooked lucerne cultivar, 'WL525' (44.67 µg/g), was however, significantly ($p<0.05$) higher than the cooked 'SAS' (25.67 µg/g). The Fe contents of the cooked 'WL711' (323.00 µg/g) and cooked SB (343.33 µg/g) were significantly ($p<0.01$) higher than the cooked 'SAS' (25.67 µg/g) and 'WL525' (176.33 µg/g) (Table 3.2).

Cooking had no significant destructive effect on the content of Ca, Mg, Zn and Fe of the three lucerne cultivars (Table 3.2). However, the K, S and Mn content of the raw lucerne samples (2.78% / 2.89% / 3.29%; 0.69% / 0.65% / 0.62%; 68.33 µg/g / 73.00 µg/g / 84.00 µg/g) were significantly ($p<0.001$) higher than the cooked samples (0.53% / 0.56% / 0.49%; 0.46% / 0.45% / 0.62%; 47.67 µg/g / 49.33 µg/g / 60.33 µg/g). The Mg content for the raw SB (1.52%) cultivar was also significantly ($p<0.001$) higher than the cooked SB (0.66%). The Na content of the cooked 'SAS' (1047.00 µg/g) was significantly ($p<0.001$) higher than the raw 'SAS' (464.33 µg/g). Although not significant, the Fe content of all three lucerne cultivars and the SB also increased with cooking (Table 3.2). Lee and Clydesdale (1981) confirmed that the bioavailability of Fe in vegetables may increase by cooking, however, Mepba et al. (2007) found that blanching and cooking caused significant ($p\leq0.05$) reductions in the K, Na, Ca, Zn, Fe and P contents of amaranths, tomatoes, fluted pumpkin gnectum vegetable, spinach, slippery vine and cocoyam leaves.

In Table 3.3 the proposed average mineral levels for the cooked lucerne cultivars are provided. According to the authors' knowledge, no range of mineral levels for cooked lucerne is available in SA.

3.3.4 Mineral cooking losses

Table 3.3

The combined average macro- (%) and micro-mineral (µg/g) levels in cooked 'SA Standard', 'WL525' and 'WL711' lucerne cultivars.

Mineral	Unit	Normal range
Phosphorus (P)	%	0.38 – 0.43
Potassium (K)	%	0.49 – 0.56
Calcium (Ca)	%	1.96 – 2.15
Sulphur (S)	%	0.45 – 0.46
Magnesium (Mg)	%	0.32 – 0.35
Zinc (Zn)	µg/g	25.67 – 44.67
Iron (Fe)	µg/g	176.33 – 323.00
Manganese (Mn)	µg/g	47.67 – 60.33
Sodium (Na)	µg/g	661.00 – 1047.00
Copper (Cu)	µg/g	13.33 – 16.00

Table 3.4

The average cooking losses of the minerals of the three lucerne cultivars and the spinach beet.

Mineral[1]	Unit	Spinach beet	'SA Standard'	'WL525'	'WL711'
P	%	0.151	0.065	0.073	0.112
K	%	+0.806[2]	+0.810	+0.816	+0.803
Ca	%	+0.193	+0.170	+0.194	+0.224
S	%	+0.299	+0.341	+0.348	+0.345
Mg	%	+0.322	+0.351	+0.360	+0.340
Zn	µg/g	0.032	+0.372	+0.237	+0.102
Fe	µg/g	+0.351	0.076	0.068	+0.098
Mn	µg/g	+0.324	+0.302	+0.291	+0.295
Na	µg/g	0.248	1.255	0.932	0.538
Cu	µg/g	0.126	+0.011	0.021	0.078

[1] = Triplicate results were calculated on a wet weight basis for each individual sample and then averaged.

[2] = A plus sign indicates a gain after cooking.

P = phosphorus; K = potassium; Ca = calcium; S = sulphur; Mg = magnesium; Zn = zinc; Fe = iron; Mn = manganese; Na = sodium; Cu = copper.

In Table 3.4 the average mineral cooking losses in lucerne and SB are provided. After cooking, all the lucerne cultivars showed a positive gain for K (+0.810 / +0.816 / +0.803), Ca

(+0.170 / +0.194 / +0.224), S (+0.341 / +0.348 / +0.345), Mg (+0.351 / +0.360 / +0.340) and Mn (+0.302 / +0.291 / +0.295). While all the lucerne cultivars showed no gain for P (0.065 / 0.073 / 0.112) and Na (1.255 / 0.932 / 0.538), SB indicated no gain for Zn (0.032). Lucerne cultivar 'WL711' and SB showed a positive gain for Fe (+0.098 / +0.351). Only lucerne cultivar 'SAS' indicated a positive gain for Cu (+0.011) (Table 3.4). According to Lisiewska, Gębczyński, Bernaś and Kmiecik (2009) and Kimura and Itokawa (1990), conditions of thermal treatment did not affect the level of mineral constituents in vegetables.

3.3.5 Dry matter, moisture, ash, fat, fibre, carbohydrates, energy, protein and amino acids contents

The DM content of both raw and cooked 'SAS' (4.91% / 12.38%) were significantly (p<0.001) higher than raw 'WL525' (21.07%), 'WL711' (21.30%) and SB (8.74%) (Table 3.5). Both raw and cooked 'WL525' (21.07% / 11.34%) and 'WL711' (21.30% / 11.79%) had a significantly (p<0.001) higher DM content than raw and cooked SB (8.74% / 8.81%). According to PBPM (2011), freshly cut lucerne will normally have a DM content between 15 – 25% which is in agreement with the results in this study. The raw SB (91.52%) had a significantly (p<0.001) higher moisture content than all the raw lucerne cultivars (71.31% / 74.61% / 73.60%) (Table 3.5). The raw 'WL525' (74.61%) had a significantly (p<0.001) higher moisture content than raw 'SAS' (71.31%).

The raw 'SAS' (3.19%) had a significantly (p<0.001) higher ash content than raw 'WL525' (2.20%), 'WL711' (2.23%) and SB (1.72%) (Table 3.5). Raw 'WL525' (2.20%) and 'WL711' (2.23%) had significantly (p<0.001) higher ash contents than SB (1.72%). These results were in agreement with Aganga and Tshwenyane (2003) and Duke (1983) who found green forage of lucerne generally contains 2.4 g ash/100 g. Milić et al. (2011), however, investigated the chemical composition of five lucerne cultivars and found an average ash value of 11 g/100 g.

The raw 'WL711' (0.51%) had a significantly (p<0.001) higher fat content than raw 'SAS' (0.35%), 'WL525' (0.39%) and SB (0.24%) (Table 3.5). The raw 'SAS' (0.35%) and 'WL525' (0.39%) had significantly (p<0.001) higher fat contents than raw SB (0.24%). Cooked 'SAS' (0.74%) had a significantly (p<0.001) higher fat content than cooked 'WL525' (0.49%), 'WL711' (0.38%) and SB (0.17%). Cooked 'WL525' (0.49%) and 'WL711' (0.38%) had significantly (p<0.001) higher fat contents than SB (0.17%) (Table 3.5). The lucerne cultivars in this study had lower fat contents than found by Aganga and Tshwenyane (2003) and Katić

Table 3.5

The dry matter, moisture, ash, fat, fibre, carbohydrate, energy and protein contents of the three lucerne cultivars and the spinach beet (% wet weight basis ± standard deviation).

Cultivar	Dry Matter %	Moisture %	Ash %	Fat %	Fibre %	Carbohydrates %	Energy (kJ/100 g)	Protein %
Spinach beet: Raw	8.74 ± 0.01[a]	91.52 ± 0.48[c]	1.72 ± 0.01[b]	0.24 ± 0.04[a]	0.66 ± 0.03[a]	4.69 ± 0.06[a]	131.00 ± 2.65[a]	2.09 ± 0.03[a]
Spinach beet: Cooked	8.81 ± 0.05[a]	91.58 ± 0.79[c]	0.97 ± 0.02[a]	0.17 ± 0.03[a]	0.92 ± 0.03[ab]	4.61 ± 0.09[a]	163.67 ± 1.53[b]	3.05 ± 0.05[b]
'SA Standard': Raw	24.91 ± 0.02[f]	71.31 ± 0.52[a]	3.19 ± 0.02[d]	0.35 ± 0.02[b]	6.47 ± 0.71[e]	16.75 ± 0.11[e]	421.00 ± 2.00[f]	4.61 ± 0.09[e]
'SA Standard': Cooked	12.38 ± 0.22[d]	89.19 ± 1.03[c]	0.80 ± 0.06[a]	0.74 ± 0.04[d]	1.63 ± 0.08[bc]	6.80 ± 0.25[c]	247.33 ± 1.15[d]	4.04 ± 0.05[c]
'WL525':Raw	21.07 ± 0.01[e]	74.61 ± 2.69[b]	2.20 ± 0.01[c]	0.39 ± 0.01[b]	5.69 ± 0.07[de]	13.96 ± 0.01[d]	356.33 ± 11.59[e]	4.53 ± 0.02[e]
'WL525': Cooked	11.34 ± 0.14[b]	89.81 ± 0.36[c]	0.87 ± 0.20[a]	0.49 ± 0.04[c]	1.48 ± 0.03[bc]	6.07 ± 0.06[b]	227.67 ± 0.58[c]	3.91 ± 0.07[c]
'WL711': Raw	21.30 ± 0.02[e]	73.60 ± 0.46[ab]	2.23 ± 0.08[c]	0.51 ± 0.03[c]	5.52 ± 0.33[d]	13.91 ± 0.07[d]	365.00 ± 11.79[e]	4.65 ± 0.02[e]
'WL711': Cooked	11.79 ± 0.15[c]	89.79 ± 0.46[c]	0.93 ± 0.15[a]	0.38 ± 0.02[b]	1.76 ± 0.05[c]	6.23 ± 0.37[b]	237.00 ± 1.73[cd]	4.24 ± 0.10[d]
Sign. level	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$

Means with different superscripts in the same column differ significantly.

et al. (2009), who reported a general value of 0.9% and 1.78%, respectively, for lucerne cultivars. However, the results of this study were in agreement with Uusiki, Oelofse, Duodu, Bester and Faber (2010), who investigated the fat content of leafy vegetables (e.g. *Amaranthus* sp., *Brassica* sp., *Spinacea oleracea*) which ranged between 0.2 – 2.2 %.

The raw 'SAS' (6.47%) had a significantly ($p<0.001$) higher fibre content than raw 'WL711' (5.52%) and SB (0.66%) (Table 3.5). The cooked 'WL711' (1.76%) had a significantly ($p<0.001$) higher fibre content than cooked SB (0.92%). These results were in agreement with other studies. Khanum, Siddalinga Swamy, Sudarshana Krishna, Santhanam and Viswanathan (2000) determined the fibre content of well-known fresh vegetables (such as amaranthus, cabbage, cauliflower and SB) and found that the fibre content ranged from 4.3 – 8.2%. Aganga and Tshwenyane (2003) found green forage of lucerne to contain 3.5 g fibre/100 g.

Both the raw and cooked 'SAS' (16.75% / 6.80%) had significantly ($p<0.001$) higher carbohydrate contents than both raw and cooked 'WL525' (13.96% / 6.07%), 'WL711' (13.91% / 6.23%) and SB (4.69% / 4.61%) (Table 3.5). Raw and cooked 'WL525' (13.96% / 6.07%) and 'WL711' (13.91% / 6.23%) had significantly ($p<0.001$) higher carbohydrate contents than SB (4.69% / 4.61%). According to Uusiki, Oelofse, Duodu, Bester and Faber (2010), the carbohydrate content for leafy vegetables (e.g. *Amaranthus* sp., *Brassica* sp., *Spinacea oleracea*) ranged from 1 – 18%. Martinson, Jung, Hathaway and Sheaffer (2012) reported a carbohydrate range of 8.7% – 13.2% for lucerne, which is in agreement with the results in this study.

The raw 'SAS' (421.00 kJ/100g) had a significantly ($p<0.001$) higher energy content than raw 'WL525' (356.33 kJ/100g), 'WL711' (365.00 kJ/100g) and SB (131.00 kJ/100g) (Table 3.5). The raw and cooked 'WL525' (356.33 kJ/100g / 227.67 kJ/100g) and 'WL711' (365.00 kJ/100g / 237.00 kJ/100g) had significantly ($p<0.001$) higher energy contents than SB (131.00 kJ/100g / 163.67 kJ/100g). Cooked 'SAS' (247.33 kJ/100g) had a significantly ($p<0.001$) higher energy content than cooked 'WL525' (227.67 kJ/100g) and SB (163.67 kJ/100g) (Table 3.5). Uusiki, Oelofse, Duodu, Bester and Faber (2010) found that the energy content for leafy vegetables (e.g. *Amaranthus* sp., *Brassica* sp., *Spinacea oleracea*) ranged from 96 – 381 kJ/100 g. Van Soest (1994) reported an energy content of 274 kJ/100 g for lucerne, which is in agreement with the results of this study.

The three raw lucerne cultivars (4.61% / 4.53% / 4.65%) had significantly ($p<0.001$) higher protein contents than raw SB (2.09%) (Table 3.5). The protein content of the raw lucerne cultivars did not differ significantly between each other, which was in agreement with Tongel and Ayen (2010). The cooked 'WL711' (4.24%), however, had a significantly ($p<0.001$) higher protein content than cooked 'SAS' (4.04%), 'WL525' (3.91%) and SB (3.05%). Cooked 'SAS' (4.04%) and 'WL525' (3.91%) had significantly ($p<0.001$) higher protein contents than cooked SB (3.05%) (Table 3.5). These results were in agreement with Aganga and Tshwenyane (2003), who reported green forage of lucerne to contain 5.2 g protein/100 g. Uusiki, Oelofse, Duodu, Bester and Faber (2010) found protein content of green leafy vegetables ranged from 1 – 7%. The protein content of the three lucerne cultivars in this study were satisfactory and compared good to the SB.

In Table 3.6, no significant differences were found for the AA of all three lucerne cultivars, except for aspartic acid (Asp) content, that was significantly ($p<0.01$) higher for raw 'SAS' (0.77 g/100g). 'SAS' indicated the highest levels of all AA evaluated in this study. Comparing the AA content of the three lucerne cultivars to the content of SB, the lucerne cultivars had higher AA contents for all the essential AA (His, Ile, Leu, Lys, Met, Phe and Val), and non-essential Arg and Thr. These results were in agreement with the results of the study on lucerne by Steward, Thompson, Millar, Thomas and Hendricks (1951).

Amino acid scoring provides a way to predict how efficiently a protein will meet a person's AA needs. A reference AA scoring pattern is used, which expresses the AA requirements in mg/g of dietary protein or as percentages in an "ideal" protein (Harper & Yoshimura, 1993). Between the lucerne cultivars, 'SAS' indicated the highest AAS for all the AA (Table 3.7). Therefore, consumers will have to consume more of 'WL525', 'WL711' and SB to receive the same amount of AA than 'SAS'. The three lucerne cultivars had higher AAS in all the AA than the SB.

The LAA for 'SA Standard' was Leu (56%) (Table 3.7). The LAA for 'WL525' was Leu (40%) and Met + Cys (40%), while Met + Cys (31%) were the LAA for 'WL711'. Lys (18%) was the LAA for SB. This is in agreement with the study done by Tedeschi, Pell, Fox and Llames (2001), where low levels of Cys, His and Met were found in lucerne cultivars. Lucerne is classified as a legume which is deficient in Met and Cys. Therefore, it can be concluded that protein complementation will be necessary to ensure a more complete protein. Protein complementation is when plant protein sources are combined to achieve a better AA balance, than either would have alone. It also increases the overall quality of the protein that is consumed. For example, lucerne is low in Met and Cys, while grains like wheat contain high amounts of Met and Cys (Murano, 2003).

Table 3.6

The amino acid composition of the raw lucerne cultivars and the spinach beet (g/100 g).

Amino acid	Spinach beet[1]	'SA Standard'	'WL525'	'WL711'	Sign. Level
Essential					
His	0.04 ± 0.02	0.25 ± 0.06	0.15 ± 0.03	0.17 ± 0.05	NS
Ile	0.15 ± 0.02	0.24 ± 0.10	0.18 ± 0.06	0.18 ± 0.05	NS
Leu	0.13 ± 0.02	0.39 ± 0.17	0.28 ± 0.08	0.26 ± 0.07	NS
Lys	0.10 ± 0.02	0.39 ± 0.09	0.28 ± 0.03	0.28 ± 0.02	NS
Met	0.02 ± 0.02	0.09 ± 0.06	0.07 ± 0.02	0.06 ± 0.03	NS
Phe	0.11 ± 0.02	0.32 ± 0.12	0.24 ± 0.06	0.23 ± 0.06	NS
Val	0.11 ± 0.02	0.29 ± 0.10	0.22 ± 0.06	0.22 ± 0.04	NS
Non-essential					
Arg	0.12 ± 0.02	0.35 ± 0.12	0.25 ± 0.05	0.23 ± 0.05	NS
Ser	ND	0.27 ± 0.09	0.20 ± 0.02	0.21 ± 0.03	NS
Asp	ND	0.77 ± 0.03[b]	0.45 ± 0.09[a]	0.56 ± 0.06[a]	p<0.01
Cys	ND	0.11 ± 0.11	0.07 ± 0.06	0.05 ± 0.07	NS
Glu	ND	0.56 ± 0.21	0.36 ± 0.13	0.39 ± 0.10	NS
Gly	ND	0.25 ± 0.08	0.18 ± 0.03	0.18 ± 0.02	NS
Thr	0.08 ± 0.03	0.27 ± 0.11	0.19 ± 0.06	0.19 ± 0.06	NS
Ala	ND	0.35 ± 0.14	0.24 ± 0.06	0.26 ± 0.06	NS
Tyr	ND	0.29 ± 0.13	0.15 ± 0.09	0.19 ± 0.06	NS
Pro	ND	0.37 ± 0.24	0.26 ± 0.11	0.25 ± 0.11	NS
HO-Pro	ND	0.07 ± 0.02	0.06 ± 0.03	0.05 ± 0.02	NS

[1] = Data from USDA (2014).

His = Histidine; Ile = Isoleucine; Leu = Leucine; Lys = Lysine; Met = Methionine; Phe = Phenylalanine; Val = Valine; Arg = Arginine; Ser = Serine; Asp = Aspartic acid; Cys = Cysteine; Glu = Glutamic acid; Gly = Glycine; Thr = Threonine; Ala = Alanine; Tyr = Tyrosine; Pro = Proline; HO-Pro = HO-Proline.

Means with different superscripts in the same row for the lucerne cultivars only, differ significantly.

ND = Not detected; NS = Not significant

Table 3.7

The amino acid scores (%) for the three lucerne cultivars and the spinach beet.

Amino acid	Spinach beet	'SA Standard'	'WL525'	'WL711'
His	24	147	88	100
Ile	38	60	45	45
Leu	19	56	40	37
Lys	18	71	51	51
Met + Cys	ND	57	40	31
Phe + Tyr	ND	102	65	70
Thr	20	68	48	48
Val	22	58	44	44

His = Histidine; Ile = Isoleucine; Leu = Leucine; Lys = Lysine; Met = Methionine; Cys = Cysteine; Phe = Phenylalanine; Tyr = Tyrosine; Thr = Threonine; Val = Valine.

ND = Not determined

3.3.6 Microbial analysis

The TBC, coliform count, LAB count and mould count for the raw lucerne cultivars and the raw SB were significantly ($p<0.001$) higher than the cooked lucerne cultivars and SB (Table 3.8), due to the destruction of the microorganisms during cooking (ICMSF, 1986).

No significant differences existed when the TBC, coliform counts, LAB counts and yeast and mould counts of the raw lucerne cultivars and the raw SB were compared. The FAO (2008) reported that vegetables have a natural epiphytic flora and may be contaminated by various intentional or accidental inputs from the growing field environment. The results in this study, therefore, indicate that the lucerne cultivars and the SB were planted and treated under similar conditions and had similar epiphytic microorganisms.

The TBC for the raw lucerne cultivars ranged between log 5.45 – log 7.13 cfu/g and the raw SB had a TBC of log 1.49 cfu/g (Table 3.8). These results were in agreement with the study by Suwarno, Wittenberg and McCaughey (1999). They investigated the TBC of lucerne during wilting and reported log 5.03 cfu/g (0 h), log 5.87 cfu/g (3 h) and log 6.15 cfu/g (24 h), respectively. It was also in agreement with other studies on ready-to-eat fresh vegetables and fresh spinach (Allen et al., 2013; Johnston et al., 2005; Pianetti et al., 2008).

Table 3.8

The microbial analysis of the three raw and cooked lucerne cultivars and raw and cooked spinach beet (log cfu/g ± standard deviation).

Microbial count	Spinach beet Raw	Spinach beet Cooked	'SA Standard' Raw	'SA Standard' Cooked	'WL525' Raw	'WL525' Cooked	'WL711' Raw	'WL711' Cooked	Sign. Level
Total bacterial count (log cfu/g)	6.12 ± 0.06^{b}	1.49 ± 0.20^{a}	6.85 ± 2.86^{b}	1.10 ± 0.17^{a}	7.13 ± 0.42^{b}	1.00 ± 0.01^{a}	5.45 ± 1.55^{b}	1.20 ± 0.35^{a}	p<0.001
Coliform count (log cfu/g)	4.08 ± 0.77^{ab}	1.00 ± 0.01^{a}	4.88 ± 2.67^{b}	1.00 ± 0.01^{a}	4.67 ± 0.77^{b}	1.00 ± 0.01^{a}	2.40 ± 1.36^{ab}	1.00 ± 0.01^{a}	p<0.001
Lactic acid bacteria count (log cfu/g)	2.91 ± 0.25^{b}	1.00 ± 0.01^{a}	3.03 ± 0.81^{b}	1.00 ± 0.01^{a}	3.08 ± 0.93^{b}	1.00 ± 0.01^{a}	2.06 ± 1.34^{ab}	1.00 ± 0.01^{a}	p<0.001
Yeast count (log cfu/g)	2.60 ± 0.70	1.00 ± 0.01	3.06 ± 2.68	1.00 ± 0.01	3.61 ± 1.49	1.00 ± 0.01	2.46 ± 2.27	1.00 ± 0.01	NS
Mould count (log cfu/g)	3.21 ± 0.93^{ab}	1.00 ± 0.01^{a}	3.95 ± 1.49^{b}	1.10 ± 0.17^{a}	4.98 ± 0.82^{b}	1.00 ± 0.01^{a}	3.85 ± 1.23^{b}	1.20 ± 0.35^{a}	p<0.001

Means with different superscripts in the same row differ significantly.

NS = Not significant

Coliforms are commonly-used bacterial indicators of the sanitary quality of foods and water (Goja & Mahmoud, 2013; Halablab et al., 2011). Coliform counts for the raw lucerne cultivars and the SB ranged from log 2.4 – log 4.88 cfu/g, while no *E. coli* were detected in all the samples. Similar coliform levels were reported by Allen et al. (2013), who detected coliform counts for spinach ranging from 'not detected' to log 4.0 cfu/g. Enterobacteriaceae counts of log 6 cfu/g were reported on the standing lucerne cultivar, 'Cody' (Lin et al., 1992). The chopping of lucerne can increase coliform, yeast and mould counts. Reports of counts up to log 8.0 cfu/g were recorded; however, these counts varied between studies, plant varieties and point of sampling (FAO, 2008).

The LAB counts for the raw lucerne cultivars and raw SB ranged from log 2.06 – log 3.08 cfu/g (Table 3.8), which was lower than the counts (log 3 – log 4 cfu/g) of spinach (*Spinacea oleracea* L.) (Babic, Royb, Watada, & Wergin, 1996; Neal et al., 2010) and SB (*Beta vulgaris* var. *cicla* L.) (log 3.76 cfu/g) (Lin et al., 1992). Lactic acid bacteria are important in lucerne silage preservation, as LAB has the ability to grow at low temperatures, propagating and causing spoilage as a result of the fermentation of sugars present in the spinach leaves (Lin et al., 1992). It is, therefore, important to reduce their initial levels, in an attempt to increase the shelf-life of bagged leafy green vegetables (Babic et al., 1996). The low LAB counts of the lucerne cultivars in this study will, therefore, ensure a longer shelf-life when the fresh leaves are bagged.

No significant differences were found for the yeast counts of the raw lucerne cultivars and SB (Table 3.8). Yeast and mould counts for the raw cultivars ranged from log 2.46 – log 3.61 cfu/g and log 3.21 – log 4.98 cfu/g, respectively. According to Lin et al. (1992), high populations (log 6 – log 7 cfu/g) of moulds have been reported on lucerne. The low counts for yeasts and moulds on all the samples in this study can be a primarily reflection of the hygienic practices employed during harvesting, transport and after blanching.

The presence of TBC, coliform, LAB, yeast and mould counts in lucerne are therefore not necessarily an indicator of a hazard to consumers, as plant foods are naturally associated with microorganisms (SADoH, 2000). However, microbial counts on lucerne cultivars can still be reduced upon harvest. Immediate surface sanitation and rapid cooling can slow product metabolism and growth of spoilage microbes (Barth et al., 2009). According to the FAO (2008), washing of the plants after harvest will not eliminate contamination – therefore, minimizing the potential for contamination in the field from the seed onward, is key to assuring microbiological quality (FAO, 2008). Overall, the raw lucerne cultivars and raw SB samples in this study were of excellent microbial quality.

3.3.7 Microbial shelf-life

An increase in the TBC, yeast count and mould count of the cooked cultivars were evident from day 0 to day 12 (Table 3.9). The TBC for the cooked cultivars ranged from log 1.20 – log 7.94 cfu/g. The TBC for 'WL711' and the SB were significantly ($p<0.001$) lower than the 'SAS' and 'WL525' cultivars from day 5 (log 1.77 cfu/g and log 2.61 cfu/g, respectively) until day 12 (log 5.96 cfu/g and log 6.54 cfu/g, respectively).

These results indicated that these cooked cultivars could have the longest shelf-life during a 12 day storage time at 4 °C. The New Zealand Food Safety Authority (NZFSA, 2005) established guidelines for the microbiological examination of ready-to-eat foods that have been cooked and then further stored. The satisfactory, marginal and unsatisfactory standard plate count (SPC) levels were < log 6 cfu/g, < log 7 cfu/g and ≥ log 7 cfu/g, respectively. When comparing the results of this study to these, 'WL711' (log 1.49 cfu/g – log 5.96 cfu/g) and SB (log 2.58 cfu/g – log 6.54 cfu/g) indicated satisfactory TBC levels up to day 12. This is in contrast with the other cultivars that indicated marginal TBC levels from day 10 and onwards.

The yeast and mould count ranged from log 1.00 – log 3.82 cfu/g and log 1.00 – log 3.56 cfu/g, respectively for the three cultivars and the SB (Table 3.9). Cultivar 'WL711' (log 5.95 cfu/g) had a significantly lower ($p<0.001$) yeast count than the other two lucerne cultivars (log 7.94 cfu/g / log 7.86 cfu/g) and the SB (log 6.54 cfu/g) on day 12. For the mould count, 'WL711' (log 1.66 cfu/g / log 2.59 cfu/g) had a significantly lower ($p<0.001$) count on days 10 and 12 than the other lucerne cultivars (log 2.70 cfu/g / log 2.64 cfu/g; log 3.52 cfu/g; log 3.56 cfu/g). According to HPA (2009), yeasts may cause spoilage at slightly lower levels (log 6 – log 7 cfu/g) in ready-to-eat foods, due to acid and gas production.

The TBC (log 7.94 cfu/g), yeast (log 3.82 cfu/g) and mould count (log 3.52 cfu/g) at day 12 for 'SAS' was significantly ($p<0.001$) higher than the other two lucerne cultivars (log 7.94 cfu/g / log 3.82 cfu/g / log 3.52 cfu/g; log 7.86 cfu/g / log 3.71 cfu/g / log 3.56 cfu/g) and the SB (log 6.54 cfu/g / log 3.44 cfu/g / log 3.02 cfu/g). Two possible explanations for these results are that: 1) the 'SAS' cultivar had more nutrients available than the other two cultivars for microorganisms to grow rapidly after cooking; or 2) 'WL711' and 'WL525' had higher antimicrobial properties than 'SAS'. Doss, Parivuguna, Vijayasanthi and Surendran (2011) investigated the antimicrobial activity of lucerne against microbial pathogens, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. It was suggested that plant steroids

Table 3.9.

The microbial shelf-life of the three cooked lucerne cultivars and the cooked spinach beet (log cfu/g ± standard deviation).

Day	Cultivar	Total bacterial count (log cfu/g)	Yeast count (log cfu/g)	Mould count (log cfu/g)
0	Spinach beet	2.58 ± 0.10[bcd]	1.00 ± 0.01[a]	1.00 ± 0.01[a]
	'SA Standard'	2.00 ± 0.22[ab]	1.00 ± 0.01[a]	1.00 ± 0.01[a]
	'WL525'	1.20 ± 0.17[a]	1.00 ± 0.01[a]	1.00 ± 0.01[a]
	'WL711'	1.49 ± 0.20[ab]	1.00 ± 0.01[a]	1.00 ± 0.01[a]
3	Spinach beet	2.57 ± 0.16[bcd]	1.00 ± 0.01[a]	1.00 ± 0.01[a]
	'SA Standard'	2.33 ± 0.19[ab]	1.00 ± 0.01[a]	1.26 ± 0.45[ab]
	'WL525'	1.43 ± 0.74[ab]	1.00 ± 0.01[a]	1.00 ± 0.01[a]
	'WL711'	1.62 ± 0.28[ab]	1.00 ± 0.01[a]	1.00 ± 0.01[a]
5	Spinach beet	2.61 ± 0.07[bcd]	1.00 ± 0.01[a]	1.00 ± 0.01[a]
	'SA Standard'	3.74 ± 0.09[de]	1.20 ± 0.17[a]	1.20 ± 0.35[ab]
	'WL525'	3.77 ± 0.29[de]	1.00 ± 0.01[a]	1.10 ± 0.17[ab]
	'WL711'	1.77 ± 0.07[ab]	1.00 ± 0.01[a]	1.00 ± 0.01[a]
7	Spinach beet	3.60 ± 0.48[cde]	1.16 ± 0.28[a]	1.32 ± 0.28[ab]
	'SA Standard'	5.12 ± 0.30[fg]	2.27 ± 0.13[b]	2.70 ± 0.12[c]
	'WL525'	5.15 ± 0.96[fg]	1.46 ± 0.41[a]	1.46 ± 0.45[ab]
	'WL711'	2.47 ± 0.39[abc]	1.00 ± 0.01[a]	1.00 ± 0.01[a]
10	Spinach beet	6.22 ± 0.55[gh]	1.41 ± 0.71[a]	2.71 ± 0.12[c]
	'SA Standard'	6.79 ± 0.17[hi]	3.02 ± 0.03[cde]	2.70 ± 0.05[c]
	'WL525'	6.58 ± 0.50[h]	2.97 ± 0.08[bcd]	2.64 ± 0.16[c]
	'WL711'	4.39 ± 0.60[ef]	1.26 ± 0.45[a]	1.66 ± 0.10[b]
12	Spinach beet	6.54 ± 0.09[h]	3.44 ± 0.22[def]	3.02 ± 0.11[cd]
	'SA Standard'	7.94 ± 0.09[i]	3.82 ± 0.09[f]	3.52 ± 0.14[d]
	'WL525'	7.86 ± 0.61[i]	3.71 ± 0.27[ef]	3.56 ± 0.20[d]
	'WL711'	5.96 ± 0.52[gh]	2.34 ± 0.31[bc]	2.59 ± 0.15[c]
Sign. Level		p<0.001	p<0.001	p<0.001

Means with different superscripts in the same column differ significantly.

steroids and tannins that were isolated from lucerne, possessed remarkable antimicrobial activity against microbial pathogens.

The South African Department of Health (2000) proposed microbiological specifications for ready-to-eat meal items. The limits for the colony count, coliform count and *E. coli* were <4 log cfu/g, <1 log cfu/g, and 0 log cfu/g, respectively. When comparing these specifications, it was evident that the cooked lucerne samples in this study were of satisfactory quality until day seven. Moreover, no *E. coli* or coliforms were detected in any of the cooked samples, which indicated good hygienic practices after cooking.

3.4 Conclusions

The chemical and microbial analysis of three lucerne cultivars and one SB cultivar (control) was performed, to determine its nutritional potential for human consumption. Lucerne, which is mainly used for animal feed, has not recently been investigated as an alternative protein source for human consumption in SA. With the evaluation of the soil for lucerne establishment, it was found that enough minerals were available for sustainable lucerne production.

All the results in this study are summarized in annexure 3. It was concluded that lucerne cultivar ‘SAS’ can be used for future studies, which could lead the way for the development of novel foods, as it indicated: a good Brix value that was significantly ($p<0.001$) higher than SB; the highest values for 10 out of the 12 chemical tests, followed by ‘WL711’ and ‘WL525’; a positive gain for seven out of 10 minerals after cooking; a high protein, DM, carbohydrate and energy content; the highest level of AA of the three lucerne cultivars; the same levels of epiphytic bacteria (TBC, coliform, LAB, yeast and mould counts); and marginal to satisfactory microbial levels up to day 7 during the shelf-life study.

CHAPTER 4

DESCRIPTIVE SENSORY ANALYSIS AND CONSUMER ACCEPTABILITY OF LUCERNE (*Medicago sativa* L.)

ABSTRACT

Three lucerne cultivars ('SA Standard', 'WL711' and 'WL525') and one SB cultivar were used: firstly, to determine sensory descriptive attributes by generic descriptive analysis (GDA); and secondly, to determine consumers' acceptance (aroma, taste, mouthfeel and overall acceptability) of lucerne. Panellists generated and reached consensus for 13 (plain samples) and 15 (stew samples) descriptors, respectively, as well as definitions, references and intensities thereof. Of all the samples described, 'SASS' showed the lowest value for potato aroma, and the lowest value, of the lucerne samples, for fibrous appearance, chewy and fibrous mouthfeel, bitter taste, and bitter and metallic aftertaste. Principal component analysis (PCA) of attributes for all the lucerne cultivars was applied to identify any factors differentiating between these cultivars. Three stew samples (SB, 'SAS' and 'WL525') and plain SB displayed the more 'positive' attributes, such as soft and wet appearance, spinach beet aroma and salty taste. 'WL711 stew' and three plain samples ('SAS', 'WL525' and 'WL711') were associated with the more 'negative' descriptors, namely grassy aroma, fibrous and chewy texture, fibrous appearance, bitter taste, and bitter and metallic aftertaste. With the acceptance test, consumers' acceptance towards SB, plain and -stew, were significantly ($p<0.001$) higher than for the lucerne cultivars. 'SA Standard stew' was the most preferred lucerne cultivar, as it's numerical values for aroma, mouthfeel, taste and overall acceptability were higher than for 'WL525' and 'WL711'.

4.1 Introduction

Despite lucerne's high nutritive value, the application of ALP in food is limited, because of its poor solubility and negative sensory properties regarding colour, taste and texture (Xie et al., 2008). However, lucerne's popularity and potential for human consumption, for specific health conditions, have increased. The surge in media coverage of these health benefits increased consumer awareness. Research efforts are also focusing on identification and evaluation of lucerne with good nutritional qualities, adaptation to adverse environmental conditions, high seed yield, pest and disease resistance (Eromosele et al*.,* 2008).

Cultivar differences in aroma, appearance, mouthfeel, taste and aftertaste might influence consumer acceptability. Consumer sensory testing determines whether the consumer likes and prefers one food product to another, thus determining the acceptability thereof (Lawless & Heymann, 1998). Food acceptance, in turn, refers to palatability, hedonic tone, liking or disliking, food flavour preference and pleasantness accompanied by food consumption (Meiselman & MacFie, 1996). According to Drake (2007), sensory perception is a crucial component that determines the acceptance of a food product. Furthermore, food preference also refers to an expressed choice between two or more foods, and special techniques are used to investigate these preferences (Lawless & Heymann, 1998).

It is well established in literature that individuals vary according to the extent to which they perceive and respond to sensory information (Dunn, 1999). The act of eating involves the processing of sensory information across a range of modalities, namely vision, touch, taste and smell (Coulthard & Blissett, 2009).

In GDA, panellists are recruited and work together in a group to identify key product attributes and appropriate intensity scales. The panellists are then trained to reliably identify and score product attributes. As panellists generate the attribute terms, the resulting descriptions are meaningful to consumers, and thus, analyses provide information to model predictions of consumer acceptability. Descriptive analysis results can be analysed statistically and then represented graphically (Chapman, Lawless, & Boor, 2001). With the use of GDA, sensory profiles are obtained for activities such as: the development of standards for quality control purposes; documenting product attributes before consumer testing, to help select attributes for inclusion in consumer questionnaires; help to explain results of consumer tests; as well as tracking sensory changes and reformulation of existing products, due to ingredient changes (Lawless & Heymann, 1998; Leighton, 2007; Meilgaard, Civille, & Carr, 1991).

No sensory analysis studies have been performed on SA lucerne cultivars to determine their sensory acceptability. These cultivars could have potential for food nutritionists, food scientists and food product developers, and the commercial market. The objectives of this chapter were firstly to determine sensory descriptive attributes of three lucerne cultivars by GDA. Secondly, consumers' acceptance of lucerne was determined. This test included the same three lucerne cultivars and one SB cultivar, which were evaluated for degree of liking for aroma, taste, mouthfeel and overall acceptability. For this study it was hypothesized that lucerne could have a similar taste profile to SB, as Levy and Fox (1935) reported that lucerne is occasionally used as a substitute for spinach in SA. Consumers were also prompted to indicate their preference for eating lucerne, as well as their intention to purchase lucerne as a vegetable.

4.2 Materials and methods

4.2.1 Preparation of samples for descriptive and acceptance testing

Lucerne cultivars, 'SAS', 'WL525' and 'WL711', and SB (*Beta vulgaris* var. *cicla* L.) were harvested early in the morning, when the dew or moisture was high, as this would limit leaf loss (Dickinson et al., 2010). For the GDA of the lucerne cultivars, sampling was done during the mid-vegetative to late vegetative stage (see annexure 2). Leaves damaged by insects or diseases were avoided. The samples were transported to the sensory laboratory of the Food Science Division, Department of Microbial, Biochemical and Food Biotechnology, UFS, where the leaves were separated from the stems.

For the acceptance tests, the eight samples consisted of: plainly cooked leaves from the three lucerne cultivars and SB; and leaves from the three lucerne cultivars and SB, cooked with potatoes and onions. Lucerne and SB leaves were washed with water to remove sandy particles and dried in a hand spinning vegetable drier. According to the method used by Mepba et al. (2007), leaves were cooked in filtered water at 96 °C for 10 min. One percent NaCl was added to the cooking water, as Kimura and Itokawa (1990) found that this addition reduced the loss of mineral constituents in cooked spinach. After cooking, the leaves were drained, weighed into 125 g portions, placed in plastic bags and stored in a freezer at -10 ºC, to prevent any deterioration or microbial spoilage of the samples, until sensory evaluation was done.

Preparation of the samples was conducted under the supervision and assistance of professional sensory analysts. Prior to the day of sensory evaluation, the frozen samples were placed in a refrigerator (4 °C) to defrost. For the stew samples, grated potato (250 ml) and onion (62.5 ml) were cooked in 100 ml filtered water for 15 min. and then mixed with 125 g cooked lucerne / SB samples. All the samples were blended with a *Braun* multiquick hand blender (Braun Gmbh, Kronberg, Germany) for 1 min., to ensure a smooth appearance and consistency. Portions (40 g) were served in glass ramekins with randomized three digit codes, covered with aluminium foil and heated in a calibrated *Defy* 621 (Defy, South Africa) oven at 70 °C for 20 min., before serving (at 40 °C) to the panel.

4.2.2 Selection of descriptive sensory panel

Nine trained and experienced panel members (6 = female; 3 = male) were selected to participate in the descriptive sensory test for the lucerne and SB samples. This panel had to adhere to the inclusion criteria for this study, as described in section 4.2.5 and was requested to complete a recruitment questionnaire (see annexure 4). Furthermore, they were screened prior to participation for recognition of the basic tastes (bitter, sweet, sour, salt and umami), a colour test and a descriptive test.

4.2.3 Sample serving and training for descriptive analysis

Lucerne and SB samples were prepared as described in section 4.2.1. and the panellists received eight samples in total. For each sample a score sheet was provided for evaluation (see annexure 5). Bottled water was provided as palate cleansers, before evaluation and between sampling. All the samples were served and evaluated according to the sensory principles and methods described in the Manual on Descriptive Analysis Testing for Sensory Evaluation (Hootman, 1992).

Vocabulary development was carried out in a conference room at the Food Science Division (UFS). The assessors were asked to taste the served samples and generate as many terms as possible to describe aroma, appearance, mouthfeel, taste and aftertaste (Tables 4.1 & 4.2). Panellists were exposed to 2 h training sessions on five consecutive days, in order to develop a clear definition for each attribute identified for the tested cultivars. Panellists generated and reached consensus for 13 (plain samples) and 15 (stew samples) descriptors, respectively, as well as definitions, references and intensities thereof.

4.2.4 Sample evaluation by trained panel

A structured line scale, with appropriate anchors, ranging from zero (0), denoting not (e.g. not salty), to ten (10), denoting extreme (e.g. extremely salty), was used to evaluate the samples (see annexure 5). All the samples were evaluated in triplicate, with an hour rest period between each session, amounting to a total of three evaluation sessions. Samples were evaluated under red lights, using red coloured bulbs (wavelength between 650 – 700 nm) (Eggert & Zook, 1986), to mask any differences in the colour of the samples. In order to ensure reliability and validity of the results, all the samples were randomly assigned.

4.2.5 Consumer panels

In order to reach the objectives of this study, specific inclusion criteria were set to which panellists had to comply. Both male and female panellists, aged 18 – 65 years, from Bloemfontein City, Free State Province (FSP), were included (Table 4.3). Panellists should have been regular users of green leafy vegetables and not have any food allergies. Furthermore, panellists should not have been pregnant or breastfeeding, or using any anti-coagulant medication. Panellists were recruited at public locations, such as the UFS in Bloemfontein, FSP. Panellists were provided with a recruitment document (see annexure 6),

Table 4.1

Attributes, definitions, references and intensities used by members of the trained sensory panel, to evaluate plainly cooked lucerne and spinach beet samples.

Attribute	Description	References and Intensities [1]
Aroma		
Grassy	Aroma associated with freshly cut grass.	Freshly cut grass (4)
Spinach	Aroma associated with cooked spinach beet.	Cooked spinach beet (2)
Appearance		
Soft	Freshly sliced mushrooms that appear not to have resistance in mouth.	Freshly sliced mushrooms (4)
Wet	The appearance of cooked spinach beet after drained.	Cooked spinach beet (4)
Fibrous	The appearance of raw spinach beet.	Raw spinach beet (7)
Mouthfeel		
Soft	Feeling of food that does not provide resistance in mouth.	*Smash* [2] (3)
Chewy	The mouthfeel of raw SB that does not disintegrate when chewed.	Raw spinach beet stem (6)
Fibrous	Amount of stringy fibers present in a SB stem.	Cooked spinach beet stem (7)
Taste		
Salty	Basic salt taste associated with NaCl.	0.15% NaCl solution (1)
Bitter	Basic bitter taste associated with caffeine.	0.02% caffeine ($C_8H_{10}N_4O_2$) solution (1)
Aftertaste		
Salty	Basic salt aftertaste associated with NaCl.	0.15% NaCl solution (1)
Bitter	Basic bitter aftertaste associated with caffeine.	0.02% $C_8H_{10}N_4O_2$ solution (1)
Metallic	Basic metallic aftertaste associated with ferrous sulphate.	0.15 % ferrous sulphate ($FeSO_4$) solution (1)

[1] = Intensities are based on a structured line scale, with appropriate anchors, ranging from zero (0), denoting not, to ten (10), denoting extreme.

[2] = Bokomo Smash Instant mash potato original.

Table 4.2

Attributes, definitions, references and intensities used by members of the trained sensory panel, to evaluate cooked lucerne and spinach beet stew samples.

Attribute	Description	References and Intensities [1]
Aroma		
Onion	Aroma associated with freshly cut onions.	Freshly cut onions (3)
Potato	Aroma associated with cooked warm potato.	Cooked warm potato (1)
Spinach	Aroma associated with cooked spinach beet.	Cooked spinach beet (2)
Grassy	Aroma associated with freshly cut grass.	Freshly cut grass (4)
Appearance		
Soft	Freshly sliced mushrooms that appear not to have resistance in mouth.	Freshly sliced mushrooms (4)
Wet	The appearance of cooked spinach beet after drained.	Cooked spinach beet (4)
Fibrous	The appearance of raw spinach beet.	Raw spinach beet (7)
Mushy	The appearance of mashed potatoes.	Overcooked (60 min.) and mashed potatoes (5)
Mouthfeel		
Mushy	The mouthfeel of mashed potatoes.	Overcooked (60 min.) and mashed potatoes (5)
Chewy	The mouthfeel of raw SB that does not disintegrate when chewed.	Raw spinach beet stem (6)
Fibrous	Amount of stringy fibers present in a SB stem.	Cooked spinach beet stem (7)
Taste		
Salty	Basic salt taste associated with NaCl.	0.15% NaCl solution (1)
Bitter	Basic bitter taste associated with caffeine.	0.02% $C_8H_{10}N_4O_2$ solution (1)
Aftertaste		
Bitter	Basic bitter aftertaste associated with caffeine.	0.02% $C_8H_{10}N_4O_2$ solution (1)
Metallic	Basic metallic aftertaste associated with ferrous sulphate.	0.15 % $FeSO_4$ solution (1)

[1] = Intensities are based on a structured line scale, with appropriate anchors, ranging from zero (0), denoting not, to ten (10), denoting extreme.

an information document (see annexure 7) and a consent form (see annexure 8) to participate in this research study.

Three hundred and eighty four consumers, 187 males and 197 females, participated in the consumer acceptance study (Table 4.3). The Statistical Consultation Unit of the Department Mathematical Statistics and Actuarial Science, UFS, Bloemfontein, recommended a representative sample size of 384, which, in turn, was representative of the population of Bloemfontein City (*n* = 369 568) in the FSP. Other variables, such as age, population group, education and income, were used to determine whether these variables could be linked to consumers' preference towards lucerne. The sample size comprised of: 1) Black African (79.16%), coloured (9.12%), Indian/Asian (2.60%) and white (9.12%) population groups; 2) R0 – R100 000 (87.24%); R101 000 – R500 000 (11.20%); R501 000 – R750 000 (0.78%); and > R750 000 income level groups; and 3) no formal education (0.26%); some primary (0.78%); primary completed (0.78%); some high (20.31%); matric (grade 12) (40.11%); technicon diploma/degree (4.43%), university degree (32.55%) and other (e.g. college or Services Sector Education and Training Authority (SSETA) diploma) (0.78%). The sample size comprised of consumers with a higher education level, i.e. consumers with 'some high' (20.31%) to 'matric (grade 12)' (40.11%) education level, as most of the consumers had to read and complete the provided questionnaires (Table 4.3). Potential panellists were also requested to complete a demographic questionnaire (see annexure 9).

4.2.6 Consumer sensory evaluation

The sensory evaluation of the lucerne and SB samples was conducted in individual booths and under red lights, to mask any colour differences between SB and lucerne. The sensory analysis facilities conformed to the American Society for Testing and Materials (Hootman, 1992) design guidelines for sensory facilities.

Each panellist received two trays for evaluation (eight samples in total) in one session. The four plainly cooked samples and four stew samples were presented separately on white trays. Bottled water, for rinsing the mouth before and between tasting of the samples, was supplied. A short questionnaire (see annexure 10) was provided to determine consumers' acceptance, preference and purchase intention towards lucerne.

To avoid fatigue, consumers first evaluated the four plainly cooked samples, followed by a ten minute break, before evaluating the four stew samples. Each sample was evaluated for

Table 4.3

Characteristics of the study population: respondents (*n* = 384).

Variable	% of Total
Location	
Bloemfontein	100.00
Gender	
Male	48.70
Female	51.30
Age (years)	
18 – 32	50.00
33 – 65	50.00
Population group	
Black African	79.16
Coloured	9.12
Indian/Asian	2.60
White	9.12
Income (Rand) per year	
R0 – 100 000	87.24
R101 000 – 500 000	11.20
R501 000 – 750 000	0.78
>R750 000	0.78
Education	
No formal education	0.26
Some primary	0.78
Primary completed	0.78
Some high	20.31
Matric (Grade 12)	40.11
Technicon diploma/degree	4.43
University degree	32.55
Other	0.78

degree of liking for aroma, taste, mouthfeel and overall acceptability on a nine-point hedonic scale (Figure 4.1). The nine structural levels ranged from 1 (‘dislike extremely’) to 9 (‘like extremely’).

Consumers were also prompted to indicate their preference for the eight samples: “*Please indicate how often you will be willing to eat the tested product*”, as well as their intention to purchase the tested product, by using a seven-point hedonic scale (Figure 4.2), ranging from 1 ('extremely unlikely') to 7 ('extremely likely'). Seven-point or nine-point hedonic scales are used to establish the acceptance of food products (Carpenter, Lyon, & Hasdell, 2000; Stone & Sidel, 2004). It is easy to use and consumers do not struggle to understand it (Moskowitz et al., 2006).

Nine-point hedonic scale

Instruction:
Please indicate with an X how much you like or dislike the product OVERALL.

Sample code: ________

Overall acceptability

1	2	3	4	5	6	7	8	9
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Fig. 4.1. Nine-point hedonic scale used for consumer acceptability study (Stone & Sidel, 2004).

Seven-point hedonic scale

Instruction:
Please indicate with a X how much it is unlikely or likely that you agree with the following statements

Sample code: ________

Purchase intention

1	2	3	4	5	6	7
Extremely unlikely	Very unlikely	Somewhat unlikely	Not sure	Somewhat likely	Very likely	Extremely likely

Fig. 4.2. Seven-point hedonic scale used for consumer purchase intention (Lawless & Heymann, 1998).

4.2.7 Statistical analysis

All the data was entered in Microsoft Excel 2010 spread sheets and statistical analyses were performed on XLSTAT 2007 (http://www.xlstat.com).

4.2.7.1 Sensory data from descriptive analysis

The significance of all the sensory attributes, measured for each lucerne and SB sample, was tested by means of ANOVA, which tested the main effects of the sample at a 5% significance level. If the main effect was significant, the Fisher protected-, t-test Least Significant Difference (LSD) was applied to separate the sample means. The interpretation of the descriptive sensory evaluation was simplified with the assistance of the multivariate statistical procedure, PCA. Principle component analysis is used to identify the smallest number of latent variables called "principal components" that explain the greatest amount of variability (Meilgaard et al., 2007). Residual errors, or the distances between the assessors' individual configurations and the consensus, were used to calculate co-ordinates for plotting the assessors, to identify outliers or groups (Jack, 1994).

4.2.7.2 Sensory data from consumer panel and the effect of consumer profiles on the hedonic ratings of lucerne and spinach beet

The significance of consumers' acceptance, preference, purchase intention and the effect of the sensory data from the consumer panel on the hedonic ratings, measured for each lucerne and SB sample, was tested by means of ANOVA. If the main effect was significant, Fisher's LSD-test ($p \leq 0.05$) was applied to determine the direction of the differences between mean values (NCSS, 2007).

4.3 Results and discussion

4.3.1 Descriptive analysis of three lucerne cultivars

The sensory descriptors for the lucerne and SB samples are presented in Table 4.4.

4.3.1.1 Grassy aroma

Grassy is defined as a green aromatic associated with newly cut grass and leafy plants, characterized by sweet and pungent characters (Talavera-Bianchi, Chambers, & Chambers, 2010). In this study the trained panel defined grassy aroma as an aroma associated with freshly cut grass (Tables 4.1 & 4.2). As indicated in Table 4.4, spinach beet plain (SBP), with the highest value of 6.04, was significantly ($p<0.001$) grassier in aroma than spinach beet stew (SBS) (0.96), 'SA Standard plain' ('SASP') (4.93), 'SA Standard stew' ('SASS') (1.44), 'WL525 plain' ('WL525P') (3.04), 'WL525 stew' ('WL525S') (1.41), 'WL711 plain' ('WL711P') (4.19) and 'WL711 stew' ('WL711S') (1.89); SBS had the lowest value of 0.96. 'SA Standard plain' (4.93) was not only significantly ($p<0.001$) grassier than SBS (0.96), but also grassier than 'SASS' (1.44), 'WL525P' (3.04), 'WL525S' (1.41), 'WL711P' (4.19) and 'WL711S' (1.89). Values for grassy aroma for 'SASS' (1.44) was numerical higher than SBS (0.96) and

‘WL525S’ (1.41), but significantly (p<0.001) less grassy than ‘WL525P’ (3.04) and ‘WL711P’ (4.19); ‘SASS’ (1.44) was numerical lower than ‘WL711S’ (1.89). ‘WL525 plain’ (3.04) was significantly (p<0.001) grassier in aroma than SBS (0.96), ‘WL525S’ (1.41) and ‘WL711S’ (1.89), but significantly (p<0.001) less grassy than ‘WL711P’ (4.19). Values for grassy aroma for ‘WL525S’ (1.41) was numerical higher than SBS (0.96), but significantly (p<0.001) less grassy than ‘WL711P’ (4.19) and numerical lower than ‘WL711S’ (1.89). ‘WL711 plain’ (4.19) was significantly (p<0.001) grassier in aroma than SBS (0.96), ‘WL525P’ (3.04) and ‘WL711S’ (1.41). ‘WL711 stew’ (1.89) was significantly (p<0.001) grassier than SBS (0.96) (Table 4.4).

The grassy aroma for the lucerne plain (4.93 / 3.04 / 4.19) samples were significantly (p<0.001) higher than the lucerne stew (1.44 / 1.41 / 1.89) samples. This was because the potato and onion in the stew samples disguised the grassy aroma. When comparing the lucerne cultivars, ‘SASP’ (4.93) was the grassiest and ‘WL525S’ (1.41) the least grassy (Table 4.4). Goff and Klee (2006) stated that the presence of “green” (including grassy) flavours in foods may be beneficial to human health, as they may represent “sensory cues” to the presence of free fatty acids, which are considered essential to the human diet. According to Adamczyk et al. (2010), the term “green” and similar terms such as “grassy” have been referenced recently in many descriptive sensory analyses of various foods, including tomatoes (Baldwin, Goodner, Plotto, Pritchett, & Einstein, 2004), bean products (Vara-Ubol, Chambers, & Chambers, 2004), virgin olive oil (Morales, Aparicio, & Calvente, 1996) and durian (Voon et al., 2007). The identified grassy aroma could be due to the presence of hexanal (Buettner & Mestres, 2005; Hongsoongnern, 2007; Reiners & Grosch, 1998), which, according to Buttery, Kamm and Ling (1982), is present in lucerne. Furthermore, Näf and Velluz (2000), investigating the volatile constituents of spinach leaves (*Spinacia oleracea* L.), also identified hexanal compounds.

4.3.1.2 Spinach aroma

Spinach aroma can be defined as the brown, green, slightly musty, earthy aromatics associated with fresh spinach (Talavera-Bianchi et al., 2010). Panellists defined spinach aroma as an aroma associated with the smell of cooked spinach beet (Tables 4.1 & 4.2). Spinach beet plain (4.00) was significantly (p<0.001) higher in spinach aroma than ‘SASP’ (2.37), ‘WL525P’ (1.81) and ‘WL711P’ (1.48); it was also numerical higher in spinach aroma than ‘SASS’ (3.74), ‘WL525S’ (3.56) and ‘WL711S’ (3.56), but significantly (p<0.001) less than SBS (6.26). In turn, SBS had the highest value and was significantly (p<0.001) higher

Table 4.4

Sensory descriptors for spinach beet and lucerne samples.

	SBP	SBS	'SASP'	'SASS'	'WL525P'	'WL525S'	'WL711P'	'WL711S'	Sign. Level
Aroma									
Grassy	6.04 ± 0.34[f]	0.96 ± 0.59[a]	4.93 ± 0.73[e]	1.44 ± 0.64[ab]	3.04 ± 0.81[c]	1.41 ± 0.89[ab]	4.19 ± 0.48[d]	1.89 ± 0.75[b]	p<0.001
Spinach	4.00 ± 0.48[c]	6.26 ± 0.45[d]	2.37 ± 0.49[b]	3.74 ± 0.76[c]	1.81 ± 0.68[a]	3.56 ± 0.70[c]	1.48 ± 0.51[a]	3.56 ± 0.80[c]	p<0.001
Onion	ANU	4.19 ± 0.68[c]	ANU	2.22 ± 0.75[b]	ANU	4.22 ± 0.51[c]	ANU	1.56 ± 0.64[a]	p<0.001
Potato	ANU	1.78 ±0.64[c]	ANU	1.19 ± 0.88[ab]	ANU	1.41 ± 0.69[bc]	ANU	0.74 ± 0.81[a]	p<0.001
Appearance									
Soft	5.96 ± 0.19[e]	7.67 ± 0.55[f]	4.00 ± 0.01[b]	5.70 ± 0.72[e]	4.11 ± 0.32[bc]	5.22 ± 0.42[d]	2.89 ± 0.80[a]	4.44 ± 0.58[c]	p<0.001
Wet	5.00 ±0.28[c]	6.81 ± 0.79[d]	3.37 ± 0.49[a]	5.26 ± 0.59[c]	4.37 ± 0.56[b]	5.19 ± 0.40[c]	4.04 ± 0.81[b]	5.26 ± 0.45[c]	p<0.001
Fibrous	4.15 ± 0.36[b]	1.52 ± 0.70[a]	7.56 ± 0.51[e]	5.15 ± 0.72[c]	7.56 ± 0.51[e]	5.74 ± 0.81[d]	7.81 ± 0.40[e]	7.59 ± 0.50[e]	p<0.001
Mushy	ANU	7.63 ± 0.49[b]	ANU	5.48 ± 0.51[a]	ANU	5.41 ± 0.50[a]	ANU	5.22 ± 0.42[a]	p<0.001
Mouthfeel									
Soft	6.11 ± 0.32[b]	ANU	3.37 ± 0.49[a]	ANU	3.26 ± 0.45[a]	ANU	3.30 ± 0.47[a]	ANU	p<0.001
Chewy	1.00 ± 0.01[b]	0.11 ± 0.32[a]	6.30 ± 0.47[ef]	2.67 ± 0.68[c]	6.22 ± 0.51[ef]	3.48 ± 0.94[d]	6.63 ± 0.49[f]	6.07 ± 0.55[e]	p<0.001
Fibrous	4.07 ± 0.27[b]	1.33 ± 0.62[a]	7.48 ± 0.51[e]	4.37 ± 0.49[b]	7.52 ± 0.51[e]	5.41 ± 0.69[c]	7.52 ± 0.51[e]	6.78 ± 0.42[d]	p<0.001
Mushy	ANU	7.44 ± 0.64[d]	ANU	5.93 ± 0.73[c]	ANU	5.26 ± 0.45[b]	ANU	4.44 ± 0.70[a]	p<0.001
Taste									
Salty	1.22 ± 0.42[ab]	2.48 ± 0.75[c]	0.81 ± 0.56[a]	2.19 ± 0.48[c]	1.41 ± 0.69[b]	1.15 ± 0.36[ab]	1.22 ± 0.42[ab]	1.41 ± 0.64[b]	p<0.001
Bitter	0.04 ± 0.19[a]	0.01 ± 0.01[a]	0.89 ± 0.32[c]	0.37 ± 0.63[ab]	1.04 ± 0.71[cd]	0.63 ± 0.79[bc]	1.37 ± 0.49[d]	0.41 ± 0.50[ab]	p<0.001
Aftertaste									
Salty	0.07 ± 0.27	ANU	0.11 ± 0.32	ANU	0.22 ± 0.42	ANU	0.19 ± 0.40	ANU	p = 0.411
Bitter	0.07 ± 0.27[a]	0.01 ± 0.01[a]	1.56 ± 0.70[c]	0.67 ± 0.73[b]	1.41 ± 0.64[c]	0.81 ± 0.74[b]	1.78 ± 0.80[c]	0.85 ± 0.72[b]	p<0.001
Metallic	0.22 ± 0.42[ab]	0.11 ± 0.32[a]	1.26 ± 0.45[d]	0.56 ± 0.75[abc]	0.93 ± 0.78[cd]	0.85 ± 0.82[cd]	0.67 ± 0.55[bc]	0.63 ± 0.69[abc]	p<0.001

Means with different superscripts in the same row differ significantly. Scale indicators: 0 = None; 10 = Extreme. SBP = spinach beet plain; SBS = spinach beet stew; 'SASP' = 'SA Standard plain'; 'SASS' = 'SA Standard stew'; 'WL525P' = 'WL525 plain'; 'WL525S' = 'WL525 stew'; 'WL711P' = 'WL711 plain'; 'WL711S' = 'WL711 stew'. ANU = Attribute not used.

in spinach aroma than all the other samples (Table 4.4). 'SA Standard plain' (2.37) was significantly ($p<0.001$) higher in spinach aroma than 'WL525P' (1.81) and 'WL711P' (1.48), but significantly ($p<0.001$) lower in spinach aroma than 'SASS' (3.74), 'WL525S' (3.56) and 'WL711S' (3.56). 'SA Standard stew' (3.74) was significantly ($p<0.001$) higher in spinach aroma than 'WL525P' (1.81) and 'WL711P' (1.48), and numerical higher than 'WL525S' (3.56) and 'WL711S' (3.56). Value for spinach aroma for 'WL525P' (1.81) was numerical higher than 'WL711P' (1.48), but significantly ($p<0.001$) less than 'WL525S' (3.56) and 'WL711S' (3.56). 'WL525 stew' (3.56) and 'WL711S' (3.56) were significantly ($p<0.001$) more spinach in aroma than 'WL711P' (1.48) (Table 4.4).

The stew (3.74 / 3.56 / 3.56) samples for the three lucerne cultivars were significantly ($p<0.001$) higher in spinach aroma than the plain lucerne (2.37 / 1.81 / 1.48) samples. For all the samples, SBS (6.26) was the most spinach in aroma and 'WL711P' (1.48) the least. When comparing the lucerne cultivars, 'SASS' (3.74) had the highest spinach aroma and 'WL711P' (1.48) the lowest (Table 4.4). Belitz, Grosch and Schieberle (2009) identified aroma compounds, such as (Z)-3-hexenal and methanethiol, which could be responsible for the spinach aroma. Sekiya and Hatanaka (1977) identified 3-hexenal compounds from linolenic and linoleic acids in lucerne, and Buttery, Kamm and Ling (1982) noted the presence of hexenal in lucerne. Reeves and Francis (1998) reported that methanetiol are generally present in lucerne forages.

4.3.1.3 Onion aroma

Onion aroma can be defined as an aromatic associated with onion (Bett, 2002). The panellists defined onion aroma as an aroma associated with freshly cut onions (Table 4.2). Spinach beet stew (4.19) was significantly ($p<0.001$) higher in onion aroma than 'SASS' (2.22) and 'WL711S' (1.56); in turn, 'WL711S' which had the lowest value, was significantly ($p<0.001$) lower in onion aroma than all the samples. 'WL525 stew', the lucerne sample with the highest value of 4.22, was significantly ($p<0.001$) higher than 'SASS' (2.22) and 'WL711S' (1.56) for onion aroma; 'SASS' (2.22) was also significantly ($p<0.001$) higher in onion aroma than 'WL711S' (1.56) (Table 4.4). The higher onion aroma in SBS can be ascribed to the low grassy aroma (0.96) identified in section 4.3.1.1 and high spinach aroma (6.26) in section 4.3.1.2, as well as the release of sulphur volatile compounds, such as dipropyl disulphide and *trans*-1-propenyl propyl disulphide, which are released in cooked onions (Blank, 2002). The lower onion aroma in lucerne samples, such as 'SASS' (2.22) and 'WL711S' (1.56), could be ascribed to the presence of a higher grassy (1.44 / 1.89) and spinach aroma (3.74 / 3.56) (Table 4.4).

4.3.1.4 Potato aroma

Potato aroma can be defined as an aromatic associated with baked potato (Leksrisompong, Whitson, Truong, & Drake, 2012). In this study, potato aroma was defined as the aroma associated with warm cooked potato (Table 4.2). For potato aroma, SBS, with the highest value of 1.78, was significantly (p<0.001) higher in potato aroma than 'SASS' (1.19) and 'WL711S' (0.74) (Table 4.4); and numerical higher than 'WL525S' (1.41). The value for potato aroma, for 'SASS' (1.19), was also numerical higher than 'WL711S' (0.74). In turn, 'WL711S', with the lowest value, was significantly (p<0.001) lower in potato aroma than 'WL525S' (1.41) and numerical lower than 'SASS' (1.19) (Table 4.4). The detection of the potato aroma could be due to the release of the volatile compounds, methional or pyrazines (e.g. 2-isopropyl-3-methoxy) (Belitz et al., 2009). The lower potato aroma in the lucerne samples, such as 'SASS' (2.22) and 'WL711S' (1.56), could be the result of a higher grassy (1.44 / 1.89) and spinach aroma (3.74 / 3.56) (Table 4.4).

4.3.1.5 Soft appearance

Lawless and Heymann (1999) stated that appearance of food products is a primary indicator of perceived quality. Panellists defined soft appearance as freshly sliced mushrooms that appear not to have resistance in the mouth (Tables 4.1 & 4.2). For soft appearance, SBP (5.96) was significantly (p<0.001) softer than 'SASP' (4.00), 'WL525P' (4.11), 'WL525S' (5.22), 'WL711P' (2.89) and 'WL711S' (4.44). In turn, SBS, with the highest value of 7.67, was significantly (p<0.001) softer than all the other samples (5.96 / 4.00 / 5.70 / 4.11 / 5.22 / 2.89 / 4.44). 'WL711 plain' (2.89) had the lowest value and was significantly (p<0.001) harder than all the other samples (Table 4.4).

'SA Standard plain' (4.00) was significantly (p<0.001) softer than 'WL711P' (2.89). The latter had significantly (p<0.001) lower values than 'SASS' (5.70), 'WL525S' (5.22) and 'WL711S' (4.44). For 'SASS' (5.70) soft appearance was significantly (p<0.001) higher than 'WL525P' (4.11), 'WL525S' (5.22), 'WL711P' (2.89) and 'WL711S' (4.44). 'WL525 plain' (4.11) was significantly (p<0.001) harder than 'WL525S' (5.22) and numerical harder than 'WL711S' (4.44); the value for 'WL711S' (4.44) was significantly (p<0.001) higher than 'WL711P' (2.89). When comparing the lucerne cultivars, 'SASS' (5.70) was the softest in appearance (Table 4.4). This description of a softer appearance could possibly be linked to the fact that 'SASS' was less fibrous in appearance (section 4.3.1.7) and mouthfeel (4.3.1.11).

4.3.1.6 Wet appearance

In the present study wet appearance was defined by the panelists as the appearance of cooked SB after being drained (Tables 4.1 & 4.2). Spinach beet plain (5.00) was significantly ($p<0.001$) wetter in appearance than 'SASP' (3.37), 'WL525P' (4.37) and 'WL711P' (4.04), but significantly ($p<0.001$) less wet than SBS (6.81). In turn, SBS, with the highest value, was significantly ($p<0.001$) wetter in appearance than all the samples.

'SA Standard plain', with the lowest value of 3.37, was significantly ($p<0.001$) less wet in appearance than all the samples. 'SA Standard stew' (5.26) and 'WL711S' (5.26) had significantly ($p<0.001$) higher values than 'SASP' (3.37), 'WL525P' (4.37) and 'WL711P' (4.04). 'WL525 plain' (4.37) was significantly ($p<0.001$) less wet than 'WL525S' (5.19) and 'WL711S' (5.26). On the other hand, 'WL525S' (5.19) had significantly ($p<0.001$) higher values for wet appearance than 'SASP' (3.37) and 'WL711P' (4.04). 'WL711 plain' (4.04) appeared significantly ($p<0.001$) less wet than 'WL711S' (5.26) (Table 4.4).

When comparing the lucerne samples, 'SASS' (5.26) and 'WL711S' (5.26) was the wettest in appearance (Table 4.4), which could be ascribed to the high moisture content of 89.19% and 89.79%, respectively, that was determined in Chapter 3 (section 3.3.5, Table 3.5).

4.3.1.7 Fibrous appearance

Fibrous appearance can be defined as the amount of stringy fibers present in the sample (Leksrisompong et al., 2012). Panellists in this study defined fibrous appearance as the appearance of raw spinach beet (Tables 4.1 & 4.2). Spinach beet plain (4.15) was significantly ($p<0.001$) more fibrous in appearance than SBS (1.52). In turn, SBS, with the lowest value, was also significantly ($p<0.001$) less fibrous in appearance than the plain and stew samples of all the lucerne cultivars. 'WL711 plain' (7.81), with the highest value, was significantly ($p<0.001$) more fibrous than SBP (4.15), SBS (1.52), 'SASS' (5.15), and 'WL525S' (5.74). 'SA Standard plain' (7.56) and 'WL525P' (7.56) were significantly ($p<0.001$) more fibrous in appearance than 'SASS' (5.15) and 'WL525S' (5.74), but numerical lower than 'WL711P' (7.81) and 'WL711S' (7.59). 'SA Standard stew' (5.15) was significantly ($p<0.001$) less fibrous than 'WL525S' (5.74), 'WL711P' (7.81) and 'WL711S' (7.59). 'WL525 stew' (5.74) was significantly ($p<0.001$) less fibrous than 'WL711P' (7.81) and 'WL711S' (7.59) (Table 4.4).

The fibrous appearance could be ascribed to the lower fiber content for 'WL711P' (1.76 %) and SBS (0.92 %), respectively, in Chapter 3 (section 3.3.5, Table 3.5). For the lucerne samples, 'SASS' (5.15) was the least fibrous in appearance with a lower fibre content of 1.63 % compared to 'WL711P', which had the highest fibrous appearance (1.76 %) in Chapter 3, Table 3.5.

4.3.1.8 Mushy appearance

In this section the panelists did not use the 'mushy appearance' attribute for the plain samples. Mushy appearance was defined as the appearance of mashed potatoes (Table 4.2). Spinach beet stew, with the highest value of 7.63, was significantly ($p<0.001$) mushier than all the samples. In turn, 'WL711S' (5.22) was significantly ($p<0.001$) less mushy in appearance than all the samples (Table 4.4).

When comparing the lucerne samples, 'SASS' (5.48) was the mushiest (Table 4.4), because it was less fibrous in appearance (section 4.3.1.7) and mouthfeel (section 4.3.1.11), and more wet in appearance (section 4.3.1.6*),* because of its high moisture content (89.19%) (Chapter 3, section 3.3.5, Table 3.5).

4.3.1.9 Soft mouthfeel

The panelists did not use the attribute 'soft mouthfeel' for the stew samples. Panellists defined soft mouthfeel as the feeling of food that did not provide resistance in the mouth, such as *Bokomo Smash* (Table 4.1). Spinach beet plain, with the highest value of 6.11, was significantly ($p<0.001$) softer than 'SASP' (3.37), 'WL711P' (3.30) and 'WL525P' (3.26) (Table 4.4). When comparing the lucerne samples, 'SASP' (3.37) had the softest mouthfeel (Table 4.4), which could be due to the higher fat content of 0.74% compared to SBP (0.17%), 'WL525P' (0.49%) and 'WL711P' (0.38%), and lower fibre content (1.63 %) compared to cooked 'WL711' (1.76%) that were determined in Chapter 3 (section 3.3.5, Table 3.5). According to Murano (2003), naturally contained fat contributes to mouthfeel and softness in vegetable products.

4.3.1.10 Chewy mouthfeel

Panellists defined chewy mouthfeel as the mouthfeel of raw SB, that did not disintegrate when chewed (Tables 4.1 & 4.2). Spinach beet plain, at 1.00, which was barely detectable, was significantly ($p<0.001$) chewier than SBS, where chewiness was not detectable (0.11). Therefore, all the other samples had significantly ($p<0.001$) chewier mouthfeel than SBS. 'SA Standard plain' (6.30) was significantly ($p<0.001$) chewier than 'SASS' (2.67) and

‘WL525S’ (3.48). However, in section 4.3.1.9, ‘SASP’ (3.37) had the softest mouthfeel. Plant foods can both be soft and chewy in mouthfeel, as described by Falquero, Pussat and Vouland (2014), who reported that water rehydrated (50 °C) vegetable (e.g. celery, spinach) cubes or pieces had a soft and chewy mouthfeel.

‘SA Standard stew’ (2.67) was significantly ($p<0.001$) less chewy than ‘WL525P’ (6.22), ‘WL525S’ (3.48), ‘WL711P’ (6.63) and ‘WL711S’ (6.07). For ‘WL525P’ (6.22), the value was significantly ($p<0.001$) chewier than ‘WL525S’ (3.48). ‘WL525 stew’ (3.48) was significantly ($p<0.001$) less chewy than ‘WL711P’ (6.63) and ‘WL711S’ (6.07). In turn, ‘WL711P’, with the highest value of 6.63, was significantly ($p<0.001$) chewier than ‘WL711S’ (6.07) (Table 4.4). When comparing the lucerne samples, ‘SASS’ (2.67) was the least chewy (Table 4.4). This result could be linked to the less fibrous mouthfeel of ‘SASS’ (4.3.1.11).

4.3.1.11 Fibrous mouthfeel

Panellists defined fibrous mouthfeel as the amount of stringy fibers present in a spinach beet stem (Tables 4.1 & 4.2). Spinach beet plain (4.07) was significantly ($p<0.001$) more fibrous than SBS, with the lowest value of 1.33; in turn, SBS was significantly ($p<0.001$) less fibrous than the plain and stew lucerne samples (Table 4.4). ‘SA Standard plain’ (7.48), was significantly ($p<0.001$) more fibrous than ‘SASS’ (4.37), ‘WL525S’ (5.41) and ‘WL711S’ (6.78). However, in section 4.3.1.9, ‘SASP’ (3.37) had the softest mouthfeel. Plant foods can both be soft and fibrous in mouthfeel, as described by Dawson (1998), who reported that cabbage (*Stilbocarpa polaris*) leaves had a soft fibrous mouthfeel. ‘WL525 plain’ and ‘WL711P’, both with the highest value of 7.52, were significantly ($p<0.001$) more fibrous than ‘WL525S’ (5.41) and ‘WL711S’ (6.78). ‘SA Standard stew’ (4.37) was significantly ($p<0.001$) less fibrous than the plain and stew samples of the other cultivars. ‘WL525 stew’ (5.41) was significantly ($p<0.001$) less fibrous than ‘WL711S’ (6.78) (Table 4.4).

When comparing the lucerne cultivars, ‘SASS’ (4.37) was the least fibrous (Table 4.4), which could also be ascribed to the fibre content of 1.63%, that was determined in Chapter 3 (section 3.3.5, Table 3.5). The fiber content of lucerne is composed of 16.2% cellulose, 19.5% hemicellulose and 7.6% lignin, which are classified as insoluble fibers (Colas et al., 2013). These insoluble fibers are not digested, thus providing stool bulk and not energy for humans (McWilliams, 2011).

4.3.1.12 Mushy mouthfeel

In this section the panelists did not use the mushy mouthfeel attribute for the plain samples. The trained panel defined mushy mouthfeel as the texture of mashed potatoes (Table 4.2). Spinach beet stew, with the highest value of 7.44, was significantly ($p<0.001$) mushier than 'SASS' (5.93), 'WL525S' (5.26) and 'WL711S' (4.44). In turn, 'WL711S', with the lowest value, was significantly ($p<0.001$) less mushy than all the samples (Table 4.4). 'SA Standard stew' (5.93) was significantly ($p<0.001$) mushier than 'WL525S' (5.26); in turn 'WL525' (5.26) was significantly ($p<0.001$) mushier than 'WL711S' (4.44).

When comparing the lucerne samples, 'SASS' (5.93) was the mushiest (Table 4.4). The mushy mouthfeel of 'SASS' could also be ascribed to the high moisture (89.19%) and low fibre content (1.63 %) that were determined in Chapter 3 (section 3.3.5, Table 3.5).

4.3.1.13 Salty taste

Panellists defined salty taste as the basic taste associated with NaCl (Tables 4.1 & 4.2). The salty taste in the samples resulted from the 1% NaCl that was added to the cooking water during the preparation of the samples, to reduce the loss of mineral constituents in cooked spinach (Kimura & Itokawa, 1990).

The detection of the salty taste was almost not recognized in the 'SASP' sample, with the lowest value of 0.81. It was also significantly ($p<0.001$) less salty than SBS (2.48), 'SASS' (2.19), 'WL525P' (1.41) and 'WL711S' (1.41). Spinach beet stew, with the highest value of 2.48, was significantly ($p<0.001$) more salty than all the samples, but did not differ significantly from 'SASS' (2.19) (Table 4.4). 'SA Standard stew' (2.19) was also significantly ($p<0.001$) saltier than 'WL525P' (1.41), 'WL525S' (1.15), 'WL711P' (1.22) and 'WL711S' (1.41). 'WL525 plain' (1.41) and 'WL711S' (1.41) were significantly ($p<0.001$) saltier than 'SASP' (0.81) (Table 4.4). When comparing the lucerne samples, 'SASS' (2.19) was the saltiest (Table 4.4). This result is contradicting to the wet appearance (5.26) in section 4.3.1.6 and high moisture content (89.19%) reported in Chapter 3 (section 3.3.5, Table 3.5) as food with increased levels in salt usually contains lower moisture levels (McWilliams, 2011). The salty taste for SBP (1.22) and 'SASP' (0.81) was significantly ($p<0.001$) higher than SBS (2.48) and 'SASS' (2.19). Different concentrations of halide salts, such as lithium chloride (LiCl) and lithium bromide (LiBr), may be present in different cultivars. According to Murphy, Cardello and Brand (1981), cations and anions contribute to the tastes of the halide salts, with the lighter anions producing saltier-tasting salts. The saltier taste of the SB and the SAS could, therefore be due to lighter anions.

4.3.1.14 Bitter taste

Bitter taste can be defined as the taste on the tongue stimulated by caffeine/quinine/alkaloids (Krinsky et al., 2006). In this study the bitter taste was defined as the basic taste associated with a 0.02% $C_8H_{10}N_4O_2$ solution (Tables 4.1 & 4.2). Spinach beet plain did not have a bitter taste at all, and with the very low value of 0.04, was significantly ($p<0.001$) less bitter than 'SASP' (0.89), 'WL525P' (1.04), 'WL525S' (0.63) and 'WL711P' (1.37). Spinach beet stew, with the lowest value of 0.01, had no bitter taste and differed significantly ($p<0.001$) from 'SASP' (0.89), 'WL525P' (1.04), 'WL525S' (0.63) and 'WL711P' (1.37).

'SA Standard plain' (0.89) was significantly ($p<0.001$) more bitter than 'SASS' (0.37) and 'WL711S' (0.41), and significantly ($p<0.001$) less bitter than 'WL711P' (1.37). In turn, 'WL711P' with the highest value of 1.37, was significantly ($p<0.001$) more bitter than all the samples. 'SA Standard stew' (0.37) was significantly ($p<0.001$) less bitter than 'WL525P' (1.04), which, in turn, was significantly ($p<0.001$) more bitter than 'WL711S' (0.41) (Table 4.4).

When comparing the lucerne samples, 'SASS' (0.37) was the least bitter (Table 4.4), possibly due to the high salt value (2.19) in section 4.3.1.13, as bitterness is suppressed by salt (Breslin, 1996). Bitter taste may be due to the presence of a bitter and throat-irritating compound, zahnic acid tridesmoside (Oleszek et al., 1992). Although low perceptions (0.01 – 1.37), were observed for all samples in the present study, the high intensity of the bitter taste, in general, should be kept in mind. Therefore, these low values are not insignificant, but only low in concentration. The higher intensity of the bitter taste could also be the consequence of a longer contact time with the tongue. Actual perception of bitterness can range between 2 – 7 sec., which is relatively a slow response compared to the other basic taste responses. Furthermore, bitterness is also often accompanied by a lingering aftertaste (Rousseff, 1990).

4.3.1.15 Salty aftertaste

In this section the panelists did not use the salty aftertaste attribute for the stew samples. For salty aftertaste, there were no significant differences and the values were extremely low (0.07 – 0.22), indicating that it could only be slightly detected in the four plain samples (Table 4.4).

4.3.1.16 Bitter aftertaste

In this study bitter aftertaste was defined as the basic bitter aftertaste associated with caffeine (Tables 4.1 & 4.2). Again, the values scored for this descriptor was fairly low, indicating that the bitterness was present in low, but detectable concentrations. Spinach beet stew, with the lowest value of 0.01, and SBP, with the value of 0.07, had no bitter aftertaste. All the other samples were significantly ($p<0.001$) more bitter in aftertaste. 'WL711 plain', with the highest value of 1.78, had the most bitter aftertaste of all the samples (Table 4.4). 'SA Standard plain' (1.56) was significantly ($p<0.001$) more bitter in aftertaste than 'SASS' (0.67), 'WL525S' (0.81) and 'WL711S' (0.85). 'SA Standard stew' (0.67) was significantly ($p<0.001$) less bitter than 'WL525P' (1.41) and 'WL711P' (1.78). The value for 'WL525P' (1.41) was significantly ($p<0.001$) higher for bitter aftertaste than 'WL525S' (0.81) and 'WL711S' (0.85) (Table 4.4).

When comparing the lucerne samples, 'SASS' (0.67) was the least bitter in aftertaste (Table 4.4). This result could be linked to results in section 4.3.1.14, where 'SASS' (0.37) was also the least bitter in taste and the saltiest (2.19) in taste (section 4.3.1.13). The prominent bitter aftertaste in 'WL711P' (1.78) could be linked to this sample also having the bitterest taste (1.37) (section 4.3.1.14). As noted earlier, bitterness is often accompanied by a lingering aftertaste (Rousseff, 1990). The bitterness in this sample could be due to the presence of the saponin, zahnic acid tridesmoside (triterpene glycoside) (Oleszek et al., 1992), which is present in the aerial parts of lucerne (*Medicago sativa* L.) (Nowacka & Oleszek, 2007).

4.3.1.17 Metallic aftertaste

Panellists defined metallic aftertaste as the basic aftertaste associated with ferrous sulphate (Tables 4.1 & 4.2). Again, the metallic aftertaste for all samples was quite low. Metallic aftertaste, after rinses with $FeSO_4$ solutions, is actually a retronasal smell. The sensation is virtually stopped, if the nose is pinched shut during tasting. Because metal salts are not volatile, this olfactory sensation probably arises from the ferrous ions catalysing a rapid lipid oxidation in the mouth, creating well-known potent odour compounds, such as 1-octen-3-one. Therefore, the detection of the metallic aftertaste at low concentration can be due to its olfactory-gustatory status. Furthermore, the perception of "metallic' may be modified by the condition of the panellists' teeth, since certain dental prostheses produce an electrolytic effect (Davis, 2003), although no panel member in the present study wore dental prostheses.

Spinach beet stew, with the lowest value of 0.11, was significantly ($p<0.001$) less metallic in aftertaste than 'SASP' (1.26), 'WL525P' (0.93), 'WL525S' (0.85), 'WL711P' (0.67). 'SA Standard plain', with the highest value of 1.26, was significantly ($p<0.001$) more metallic in aftertaste than SBP (0.22), SBS (0.11), 'SASS' (0.56), 'WL711P' (0.67) and 'WL711S' (0.63). 'WL525 plain' (0.93) and 'WL525 stew' (0.85) were significantly ($p<0.001$) more metallic in aftertaste than SBP (0.22) (Table 4.4). The metallic aftertaste for the SB and lucerne plain (0.22 / 1.26 / 0.93 / 0.67) samples were numerical higher than the SB and lucerne stew (0.11 / 0.56 / 0.85 / 0.63) samples. The combination of potato and onion in the stew samples could have disguised the metallic aftertaste.

When comparing the lucerne samples, 'SASS' (0.56) had the least metallic aftertaste (Table 4.4), due to the lower Fe content (23.13 mg/100 g) (Chapter 3, section 3.3.3, Table 3.5), as well as the higher salty taste (2.19) (Table 4.4) and lower bitter taste (0.37) (Table 4.4). According to Gillette (1985), salt can mask the metallic flavour in foods. The FHIS (2012) stated that an unpleasant aftertaste is usually blended with NaCl or other mineral salts, in order to reduce it.

A summary of the sensory descriptors for spinach beet and lucerne samples, used in this study, is presented in annexure 11. Spinach beet plain had the highest value for grassy aroma and soft mouthfeel, but the lowest value for salty taste. Spinach beet stew showed the highest values for positively associated descriptors, such as spinach aroma, potato aroma, soft and wet appearance, mushy appearance and mouthfeel, and salty taste. It had the lowest values for the negatively associated attributes such as grassy aroma, fibrous appearance, chewy and fibrous mouthfeel, bitter taste, and bitter and metallic aftertaste.

'SA Standard plain' was metallic in aftertaste, with a grassy aroma and soft mouthfeel. Furthermore, it did not have a wet appearance and neither a salty taste nor aftertaste. The 'SASS' sample was characterized by a high spinach aroma, soft, wet and mushy appearance and mouthfeel, and salty taste. On the other hand, it had a low value for potato aroma, fibrous appearance, chewy and fibrous mouthfeel, bitter taste, and bitter and metallic aftertaste.

'WL525 plain' had the highest values for grassy aroma and soft mouthfeel, but the lowest value for salty taste. 'WL525 stew' not only showed the highest value for onion aroma, but also the highest value, of the lucerne samples, for potato aroma. The highest value for fibrous appearance, chewy and fibrous mouthfeel, and bitter taste and aftertaste was given to 'WL711P', and the lowest values for spinach aroma and soft appearance.

‘WL711 stew’ was the wettest in appearance of the lucerne samples, but had the lowest onion aroma, mushy appearance and mouthfeel.

4.3.2 Principal Component Analysis (PCA)

Principal component analysis was applied to the attributes of the plain and stew SB and lucerne samples, to identify any factors differentiating between the different samples. According to Figure 4.3, dimensions 1 and 2 explained 90.54% of the variation. Of these, 72.66% was explained by dimension 1, indicating that the panel was capable of dividing the eight samples into two clusters, i.e., all the stew samples to the right side of the biplot, along with the more positively associated descriptors, such as soft, wet and mushy appearance, mushy texture, spinach, onion and potato aroma and salty taste. To the left side of the biplot, the four plain samples were grouped, with attributes that could be viewed as negative: bitter taste; bitter, salty and metallic aftertaste; fibrous and chewy texture; fibrous appearance; and grassy aroma. The last attribute on the left side of the biplot, namely soft texture, could be viewed as either positive or negative.

Dimension 2 explained a further 17.88% of the variation, dividing the descriptors into a top and bottom part. In the top part, only the two SB samples are located, along with two descriptors, soft mouthfeel and grassy aroma for the SBP, and three descriptors, soft and wet appearance, and spinach aroma for the SBS. This confirmed the results in Table 4.4, where SBP had the highest scores for soft mouthfeel and grassy aroma. Also, SBS had the highest values for soft and wet appearance and spinach aroma. All the lucerne samples were placed in the bottom part of the second dimension. The three plain samples were clustered together, along with attributes such as metallic and bitter aftertaste, bitter taste, fibrous and chewy mouthfeel; and fibrous appearance. Again this confirmed the results in Table 4.4, from which it was clear that these samples had some of the highest values for these descriptors and in some cases did not differ significantly. Although salty aftertaste was placed in the top part of the biplot, it was closer situated to ‘WL525P’ than to SBP, which is in agreement with the results in Table 4.4, where ‘WL525P’ had the highest value for this descriptor. The three lucerne stew samples were also grouped together, with attributes like mushy mouthfeel and appearance, potato and onion aroma, and salty taste. Mushy mouthfeel, and salty taste were two descriptors for which ‘SASS’ scored the highest values, while ‘WL525S’ had the highest values for potato and onion aroma. All three lucerne stew samples had fairly high values for mushy appearance (Table 4.4).

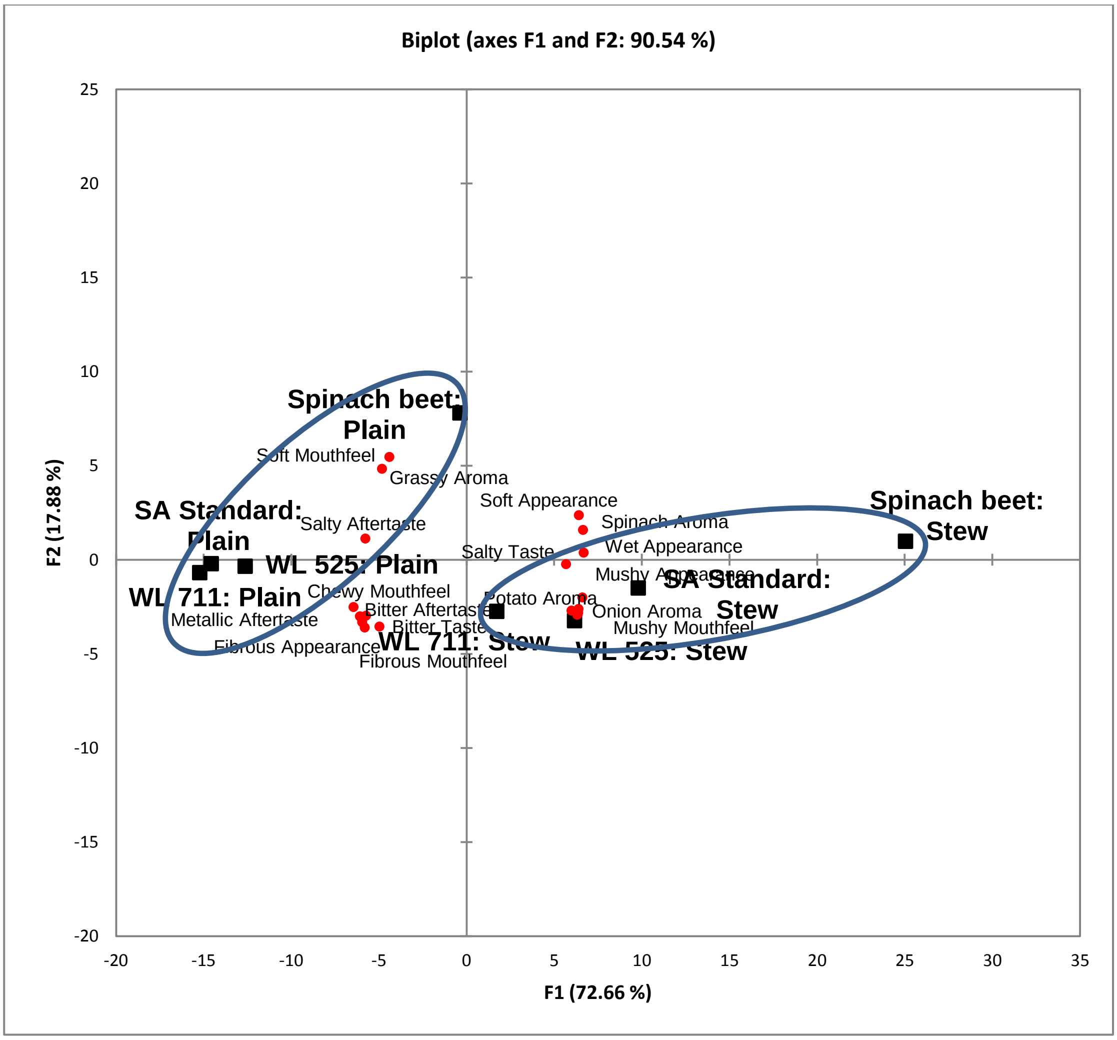
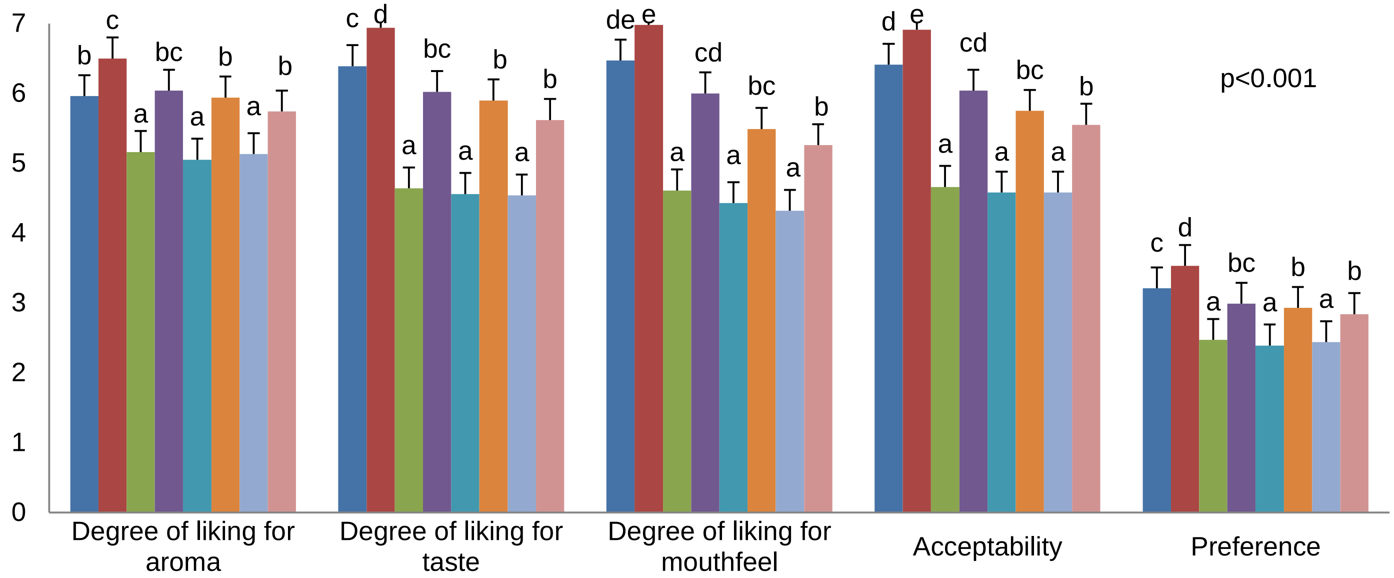


Fig 4.3. Principal Component Analysis of attributes of spinach beet and lucerne.

4.3.3 Consumer sensory acceptance, preference and purchase intention

As indicated in Figure 4.4, the degree of liking for aroma, taste, mouthfeel and overall acceptability for SBP (5.96 / 6.39 / 6.47 / 6.41) were significantly ($p<0.001$) higher than 'SASP' (5.16 / 4.64 / 4.61 / 4.66), 'WL525P' (5.05 / 4.56 / 4.43 / 4.58), and 'WL711P' (5.13 / 4.54 / 4.32 / 4.58). The degree of liking for taste, mouthfeel and overall acceptability for SBS (6.94 / 6.98 / 6.91) were also significantly ($p<0.001$) higher than 'SASS' (6.02 / 6.00 / 6.04), 'WL525S' (5.90 / 5.49 / 5.75) and 'WL711S' (5.62 / 5.26 / 5.55). The degree of liking for aroma for SBS (6.50) was significantly ($p<0.001$) higher than 'WL525S' (5.94) and 'WL711S' (5.74), but only numerical higher than 'SASS' (6.04) (Figure 4.4).

When comparing the plain lucerne cultivars, there were no significant differences for the degree of liking for aroma, taste, mouthfeel and overall acceptability. In addition, for the lucerne stew samples, no significant differences for the degree of liking for taste and aroma



	Degree of liking for aroma	Degree of liking for taste	Degree of liking for mouthfeel	Acceptability	Preference
Spinach: Plain	5.96	6.39	6.47	6.41	3.21
Spinach: Stew	6.50	6.94	6.98	6.91	3.53
SA Standard: Plain	5.16	4.64	4.61	4.66	2.47
SA Standard: Stew	6.04	6.02	6.00	6.04	2.99
WL525: Plain	5.05	4.56	4.43	4.58	2.39
WL525: Stew	5.94	5.90	5.49	5.75	2.93
WL711: Plain	5.13	4.54	4.32	4.58	2.44
WL711: Stew	5.74	5.62	5.26	5.55	2.84

Fig 4.4. Consumers' acceptance and preference of lucerne and spinach beet.

Results with different superscripts differ significantly.

Scale indicators for acceptance: 4 = dislike slightly; 5 = neither like nor dislike; 6 = like slightly; 7 = like moderately

Scale indicators for preference: 2 = I will only eat it when no other food is available; 3 = I will eat it occasionally (once per month); 4 = I will eat it often (once per week were found (Figure 4.4). The mouthfeel (6.00) and overall acceptability (6.04) for ‘SASS’ was significantly (p<0.001) higher than ‘WL711S’ (5.26 / 5.55), but not for ‘WL525S’ (5.49 / 5.75) (Figure 4.4). Although not significant, the numerical values for the degree of liking for the three sensory attributes and overall acceptability for ‘SASS’ were higher than for the other two lucerne stew samples (Figure 4.4).

For the degree of liking for aroma category, panellists ‘neither liked nor disliked’ (5) to ‘slightly liked’ (6) the plain SB (5.96) and three lucerne samples (5.16 / 5.05 / 5.13) (Figure 4.4). While the panellists ‘slightly’ (6) to ‘moderately liked’ (7) the aroma of the SBS (6.50) and ‘SASS’ (6.04), ‘WL525S’ (5.94) and ‘WL711S’ (5.74) were ‘neither liked nor disliked’ (5) to ‘slightly liked’ (6) (Figure 4.4). Not only did consumers ‘slightly (6) to moderately (7) liked’ the taste, mouthfeel and overall acceptability of the plain (6.39 / 6.47 / 6.41) and stew (6.94 / 6.98 / 6.91) SB samples, respectively, but also the ‘SASS’ sample (6.02 / 6.00 / 6.04). Although the degree of liking for taste, mouthfeel and overall acceptability for ‘SASP’ (4.64 / 4.61 / 4.66), ‘WL525P’ (4.56 / 4.43 / 4.58) and ‘WL711P’ (4.54 / 4.32 / 4.58) were ‘slightly disliked’ (4) to ‘neither liked nor disliked’ (5), the ‘WL525S’ (5.90 / 5.49 / 5.75) and ‘WL711S’ (5.62 / 5.26 / 5.55) were ‘neither liked nor disliked’ (5) to ‘slightly liked’ (6). When comparing the SBP (6.39 / 6.47 / 6.41) with ‘SASS’ (6.02 / 6.00 / 6.04), both were ‘slightly liked’ (6) to ‘moderately liked’ (7) for taste, mouthfeel and overall acceptability, except the aroma for SBP (5.96) was ‘neither liked nor disliked’ (5) to ‘slightly liked’ (6) (Figure 4.4).

When comparing the degree of liking for aroma for the plain and stew samples, SBS (6.50) was significantly (p<0.001) higher than SBP (5.96), ‘SASP’ (5.16), ‘WL525P’ (5.05), ‘WL525S’ (5.94), ‘WL711P’ (5.13) and ‘WL711S’ (5.74). Spinach beet plain (5.96), ‘SASS’ (6.04), ‘WL525S’ (5.94) and ‘WL711S’ (7.74) were significantly (p<0.001) higher than ‘SASP’ (5.16), ‘WL525P’ (5.05) and ‘WL711P’ (5.13) for degree of liking for aroma (Figure 4.4).

For the degree of liking for taste category, SBS (6.94) was significantly (p<0.001) higher than SBP (6.39), ‘SASP’ (4.64), ‘SASS’ (6.02), ‘WL525P’ (4.56), ‘WL525S’ (5.90), ‘WL711P’ (4.54) and ‘WL711S’ (5.62). Spinach beet plain (6.39) was significantly (p<0.001) higher than ‘SASP’ (4.64), ‘WL525P’ (4.56), ‘WL525S’ (5.90), ‘WL711P’ (4.54) and ‘WL711S’ (5.62). ‘SA Standard stew’ (6.02), ‘WL525S’ (5.90) and ‘WL711S’ (5.62) were significantly (p<0.001) higher than ‘SASP’ (4.64), ‘WL525P’ (4.56) and ‘WL711P’ (4.54) for degree of liking for taste (Figure 4.4).

For the degree of liking for mouthfeel category, SBS (6.98) was significantly (p<0.001) higher than ‘SASP’ (4.61), ‘SASS’ (6.00), ‘WL525P’ (4.43), ‘WL525S’ (5.49), ‘WL711P’ (4.32) and ‘WL711S’ (5.26). Spinach beet plain (6.47) was significantly (p<0.001) higher than ‘SASP’ (4.61), ‘WL525P’ (4.43), ‘WL525S’ (5.49), ‘WL711P’ (4.32) and ‘WL711S’ (5.26). ‘SA Standard stew’ (6.00) was significantly (p<0.001) higher than ‘SASP’ (4.61), ‘WL525P’ (4.43), ‘WL711P’ (4.32) and ‘WL711S’ (5.26). ‘WL525 stew’ (5.49) and ‘WL711S’ (5.26) were significantly (p<0.001) higher than ‘SASP’ (4.61), ‘WL525P’ (4.43) and ‘WL711P’ (4.32) (Figure 4.4).

For the overall acceptability category, SBS (6.91) was significantly (p<0.001) higher than SBP (6.41), ‘SASP’ (4.66), ‘SASS’ (6.04), ‘WL525P’ (4.58), ‘WL525S’ (5.75), ‘WL711P’ (4.58) and ‘WL711S’ (5.55). Spinach beet plain (6.41) and ‘SASS’ (6.04) were significantly (p<0.001) higher than ‘SASP’ (4.66), ‘WL525P’ (4.58), ‘WL525S’ (5.75), ‘WL711P’ (4.58) and ‘WL711S’ (5.55). ‘WL525 stew’ (5.75) and ‘WL711S’ (5.55) were significantly (p<0.001) higher than ‘SASP’ (4.66), ‘WL525P’ (4.58) and ‘WL711P’ (4.58) (Figure 4.4).

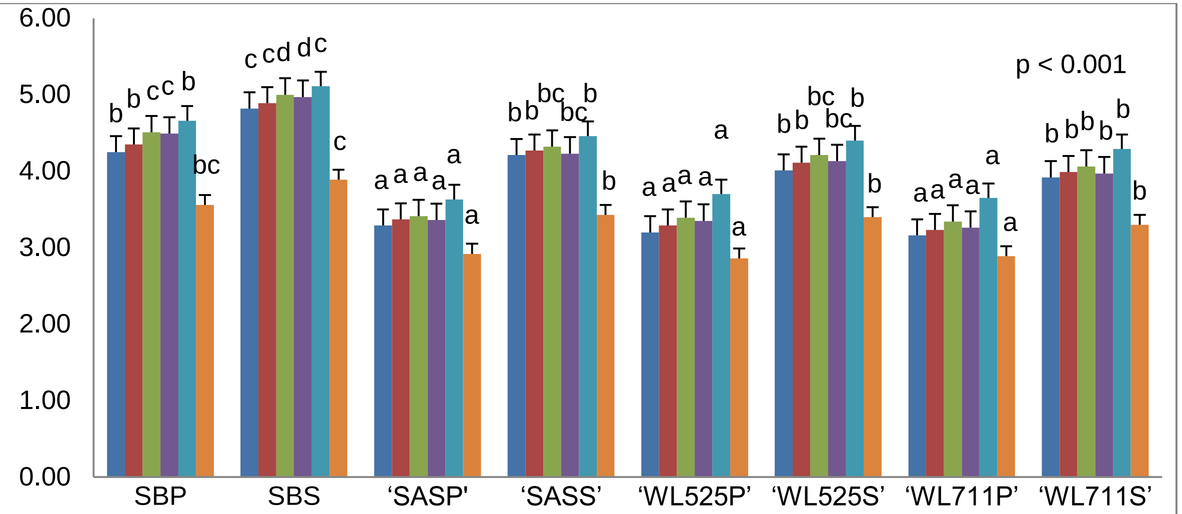
According to Figure 4.4, consumers’ preference towards SBS (3.53) were significantly (p<0.001) higher than SBP (3.21) and the plain [‘SAS’ (2.47) / ‘WL525’ (2.39) / ‘WL711’ (2.44)] and stew [‘SAS’ (2.99) / ‘WL525’ (2.93) / ‘WL711’ (2.84)] lucerne cultivars, respectively. When comparing the plain and stew samples, SBP (3.21) was significantly (p<0.001) higher than ‘SASP’ (2.47), ‘WL525P’ (2.39), ‘WL525S’ (2.93), ‘WL711P’ (2.44) and ‘WL711S’ (2.84). ‘SA Standard stew’ (2.99), ‘WL525S’ (2.93) and ‘WL711S’ (2.84) were significantly (p<0.001) higher than ‘SASP’ (2.47), ‘WL525P’ (2.39) and ‘WL711P’ (2.44). The preference for all four stew [SB (3.53) / ‘SAS’ (2.99) / ‘WL525’ (2.93) / ‘WL711’ (2.84)] samples was also significantly (p<0.001) higher than the respective plain [SB (3.21) / ‘SAS’ (2.47) / ‘WL525’ (2.39) / ‘WL711’ (2.44)] samples. Consumers indicated that they would eat both SBP (3.21) and SBS (3.53) occasionally (once per month) (3), while they would eat the lucerne samples (plain and stew) only when no other food is available (2) (Figure 4.4).

Major differences were found for the degree of liking for taste and texture, while degree of liking for aroma and overall acceptability, and preference were more closely contested. The SBS was the most preferred sample, followed by SBP and ‘SASS’ (Figure 4.4). A possible explanation for the lower acceptance towards lucerne could be due to behavioural hesitation to eat food that is originally used as animal feed in SA. Although literature has indicated that lucerne has been used for human consumption, the majority of consumers may never have consumed lucerne before. Therefore, lucerne remains an unknown food to consumers. Consequently, consumers would have less information in their memory to guide them, leading to lower preference at the time that the respondent was asked to give a judgement (Urala & Lähteenmäki, 2004).

According to Figure 4.5, consumers' purchase intention for both SBS (4.82 / 4.89 / 5.00 / 4.97 / 5.11) and SBP (4.25 / 4.35 / 4.51 / 4.49 / 4.66) was significantly ($p<0.001$) higher than for the stew and plain lucerne cultivars, respectively. Consumers indicated that they were 'not sure' (4) to 'somewhat likely' (5) that they would purchase SBP within two weeks (4.25), one month (4.35), in supermarkets (4.51) and get used to the taste of it (4.66) (Figure 4.5). Furthermore, consumers indicated that it was 'somewhat unlikely' (3), to they were 'not sure' (4), that they would purchase 'SASP' (3.29 / 3.37 / 3.41 / 3.36 / 3.63), 'WL525P' (3.20 / 3.29 / 3.39 / 3.35 / 3.70), and 'WL711P' (3.16 / 3.23 / 3.34 / 3.26 / 3.65) within two weeks, one month, in supermarkets, use it as a substitute for spinach and get used to the taste of it, respectively (Figure 4.5).

For the stew samples, consumers indicated that they were 'not sure' (4) to 'somewhat likely' (5) that they would purchase SBS within two weeks (4.82) and one month (4.89). Furthermore, consumers confirmed that they would 'somewhat likely' (5) purchase SBS in supermarkets (5.00) and it was 'very likely' (6) that they would get used to the taste of this stew (5.11). Consumers also indicated that they were 'not sure' (4) to 'somewhat likely' (5) that they would purchase 'SASS' and 'WL525S' within two weeks (4.21 / 4.01), one month (4.27 / 4.11), in supermarkets (4.32 / 4.21), use it as a substitute for SB (4.23 / 4.13) and get used to the taste of it (4.46 / 4.40), respectively. For 'WL711S', consumers said that it was 'somewhat unlikely' (3) to they were 'not sure' (4) that they would purchase it within two weeks (3.92), one month (3.99) and use it as a substitute for SB (3.97). Furthermore, consumers indicated that they were 'not sure' (4) to 'somewhat likely' (5) that they would purchase 'WL711S' in the supermarket (4.06) and get used to the taste of it (4.29) (Figure 4.5).

Figure 4.5 also shows that consumers' overall willingness to pay for a fresh 300 g packet of SB or lucerne, increased after consuming the stew samples. For the SB samples, consumers indicated that they were willing to pay between R6.00 and R15.00 for the plain (3.56) and stew samples (3.89). As the purchase amount for a SB bunch and 300 g packet SB at retailers is about R5.00 and R15.00, respectively, a hedonic scale with increments of R5.00 were used to determine consumers' purchase intention. It is interesting to note that the price of a 300 g packet of fresh SB leaves, by well-known retailers such as *Pick 'n Pay* and *Woolworths,* ranged between R16.99 and R17.99, respectively, at the time of the study. For the lucerne cultivars, consumers indicated that they were willing to pay between R1.00 and R10.00 for the plain ['SAS' (2.92) / 'WL525' (2.86) / 'WL711' (2.89)] samples, and between R6.00 and R15.00 for the stew ['SAS' (3.43) / 'WL525' (3.40) / 'WL711' (3.30)] samples (Figure 4.5).



	SBP	SBS	'SASP'	'SASS'	'WL525P'	'WL525S'	'WL711P'	'WL711S'
Likeliness to purchase tested sample within two weeks	4.25	4.82	3.29	4.21	3.20	4.01	3.16	3.92
Likeliness to purchase tested sample within one month	4.35	4.89	3.37	4.27	3.29	4.11	3.23	3.99
Likeliness to purchase tested sample in supermarkets	4.51	5.00	3.41	4.32	3.39	4.21	3.34	4.06
Likeliness to use tested sample as a substitute for spinach	4.49	4.97	3.36	4.23	3.35	4.13	3.26	3.97
Likeliness to get used to taste of tested sample	4.66	5.11	3.63	4.46	3.70	4.40	3.65	4.29
Willingness to pay for a fresh 300 g packet of tested sample	3.56	3.89	2.92	3.43	2.86	3.40	2.89	3.30

Fig 4.5. Consumers' purchase intention towards lucerne and spinach beet.

Results with different superscripts differ significantly.

Scale indicators for likeliness: 2 = very unlikely; 3 = somewhat unlikely; 4 = not sure; 5 = somewhat likely; 6 = very likely. Scale indicators for purchase amount: 2 = R1 – R5; 3 = R6 – R10; 4 = R11 – R15. SBP = spinach beet plain; SBS = spinach beet stew; 'SASP' = 'SA Standard plain'; 'SASS' = 'SA Standard stew'; 'WL525P' = 'WL525 plain'; 'WL525S' = 'WL525 stew'; 'WL711P' = 'WL711 plain'; 'WL711S' = 'WL711 stew'

Table 4.5 indicates the average gain for consumers' acceptance, preference and purchase intention between the lucerne plain and stew samples. The average gain in degree of liking for aroma for 'SAS' (0.88) and 'WL525' (0.89) were significantly ($p<0.05$) higher than SB (0.54) and 'WL711' (0.61). While the average gain in, respectively, degree of liking for taste, mouthfeel and overall acceptability for 'SAS' (1.38 / 1.39 / 1.38), 'WL525' (1.34 / 1.06 / 1.17) and 'WL711' (1.08 / 0.94 / 0.97) were significantly ($p<0.05$) higher than SB (0.55 / 0.51 / 0.50), the average gain in degree of liking for mouthfeel for 'SAS' (1.39) was significantly ($p<0.05$) higher than 'WL711' (0.94). When looking at the average gain in preference, 'SAS' (0.52) and 'WL525' (0.54) were significantly ($p<0.05$) higher than SB (0.32) and 'WL711' (0.40).

The average gain to purchase the tested sample within, respectively, two weeks, one month and at a supermarket for 'SAS' (0.92 / 0.90 / 0.91), 'WL525' (0.81 / 0.82 / 0.82) and 'WL711' (0.76 / 0.76 / 0.72) were significantly ($p<0.05$) higher than SB (0.57 / 0.54 / 0.49). Furthermore, it was observed that the average gain to purchase the tested sample within one month for 'SAS' (0.90) was significantly ($p<0.05$) higher than 'WL525' (0.82). It was also noted that the average gain to purchase the tested sample, respectively, within two weeks and in a supermarket, as well as to get used to the taste of the tested sample for 'SAS' (0.92 / 0.91 / 0.83), were significantly ($p<0.05$) higher than 'WL711' (0.76 / 0.72 / 0.64) (Table 4.5).

The average gain to, respectively, use the tested sample as a substitute for SB and get used to the taste of the tested sampled for 'SAS' (0.87 / 0.83), 'WL525' (0.78 / 0.70) and 'WL711' (0.71 / 0.64) were significantly ($p<0.05$) higher than SB (0.48 / 0.45). Furthermore, the average gain to get used to the taste of the tested sample for 'SAS' (0.83), 'WL525' (0.70) and 'WL711' (0.64) were significantly ($p<0.05$) higher than SB (0.45).

The average gain to pay for a fresh 300 g packet of sample for 'SAS' (0.51) and 'WL525' (0.54) were significantly ($p<0.05$) higher than SB (0.33) and 'WL711' (0.41) (Table 4.5). While the level of acceptance for both SBP and SBS were the same, the plain lucerne samples improved in acceptance with the addition of potato and onion. Therefore, the method of preparation and addition of ingredients played an important role to make the lucerne samples more acceptable to consumers.

Table 4.5

Average gain in consumers' acceptance, preference and purchase intention of spinach beet and lucerne plain samples versus spinach beet and lucerne stew samples.

	SB	'SA Standard'	'WL525'	'WL711'	Sign. Level
Acceptance					
Degree of liking for aroma	0.54[a]	0.88[b]	0.89[b]	0.61[a]	p<0.05
Degree of liking for taste	0.55[a]	1.38[b]	1.34[b]	1.08[b]	p<0.05
Degree of liking for mouthfeel	0.51[a]	1.39[c]	1.06[bc]	0.94[b]	p<0.05
Overall acceptability	0.50[a]	1.38[c]	1.17[bc]	0.97[b]	p<0.05
Preference	0.32[a]	0.52[b]	0.54[b]	0.40[a]	p<0.05
Purchase intention					
Likeliness to purchase tested sample within two weeks	0.57[a]	0.92[c]	0.81[bc]	0.76[b]	p<0.05
Likeliness to purchase tested sample within one month	0.54[a]	0.90[c]	0.82[b]	0.76[bc]	p<0.05
Likeliness to purchase tested sample in supermarkets	0.49[a]	0.91[c]	0.82[bc]	0.72[b]	p<0.05
Likeliness to use tested sample as a substitute for SB	0.48[a]	0.87[b]	0.78[b]	0.71[b]	p<0.05
Likeliness to get used to taste of tested sample	0.45[a]	0.83[c]	0.70[bc]	0.64[b]	p<0.05
Willingness to pay for a fresh 300 g packet of tested sample	0.33[a]	0.51[b]	0.54[b]	0.41[a]	p<0.05

Means with different superscripts in the same row differ significantly.

4.3.4 The effect of consumer profiles on the hedonic ratings of lucerne

The Fisher's LSD-test was applied to all the main effects that were significant, to determine the direction of the differences between mean values. For the main effect gender (Figure 4.6), two samples showed significant differences. Male consumers' acceptability for 'WL525P' (4.82) and 'WL711P' (4.86) were significantly ($p<0.05$) higher than for female (4.35 / 4.31) consumers (Figure 4.6). In section 4.3.1.14, it was found that 'WL525P' and 'WL711P' were more bitter than 'SASP' and SBP. This could indicate that male consumers prefer more bitter tasting foods. Demura et al. (2013) studied gender differences in coffee consumption and found the coffee consumption rate was significantly higher in males (50.8%) than in females (32.8%) and that females (64.8%) disliked its taste more than males (39.4%). However, the tendency to consume or reject bitter tasting substances varies between individuals, which are largely due to genetic differences in bitter taste receptors (El-Sohemy, 2010).

Age (Figure 4.7), as main effect, showed four significant differences. The age group, 33 – 65 years' acceptability was significantly higher for 'WL525P' (4.84) ($p<0.05$), 'WL711P' (4.96) ($p<0.01$), 'WL711S' (5.79) ($p<0.05$), and SBP (6.75) ($p<0.01$) than the 18 – 32 years age group (4.32 / 4.20 / 5.31 / 6.06) (Figure 4.7). When a comparison is made between the younger and older consumers, it is important to look at factors that may influence acceptance behaviour, such as cognitive differences and socio-economic factors (e.g. urbanization) in SA towards food products. According to Puoane, Matwa, Bradley and Gail Hughes (2006), the consumption of traditional plant foods is largely associated with poverty and consequently, as people move to the city, they change their diet to a typical Westernised diet, with a high fat content and low carbohydrate intake. Younger consumers abandon traditional grains, root plants, lentils and greens, and they usually adopt foods that are associated with status, such as meat and fast foods.

For the main effect race (Figure 4.8), two samples showed significant differences. The Black African groups' acceptability for SBP (6.59) ($p<0.01$) and SBS (7.08) were significantly ($p<0.05$) higher than the coloured group (5.49 / 6.23) (Figure 4.8). This could be explained by the fact that Black Africans, who started to settle in SA about 2 000 years ago, collected leafy vegetables from the wild (Bundy, 1988). Collecting and cultivating green leafy vegetables continues to be widespread among African people in SA (Jansen van Rensburg et al., 2007).

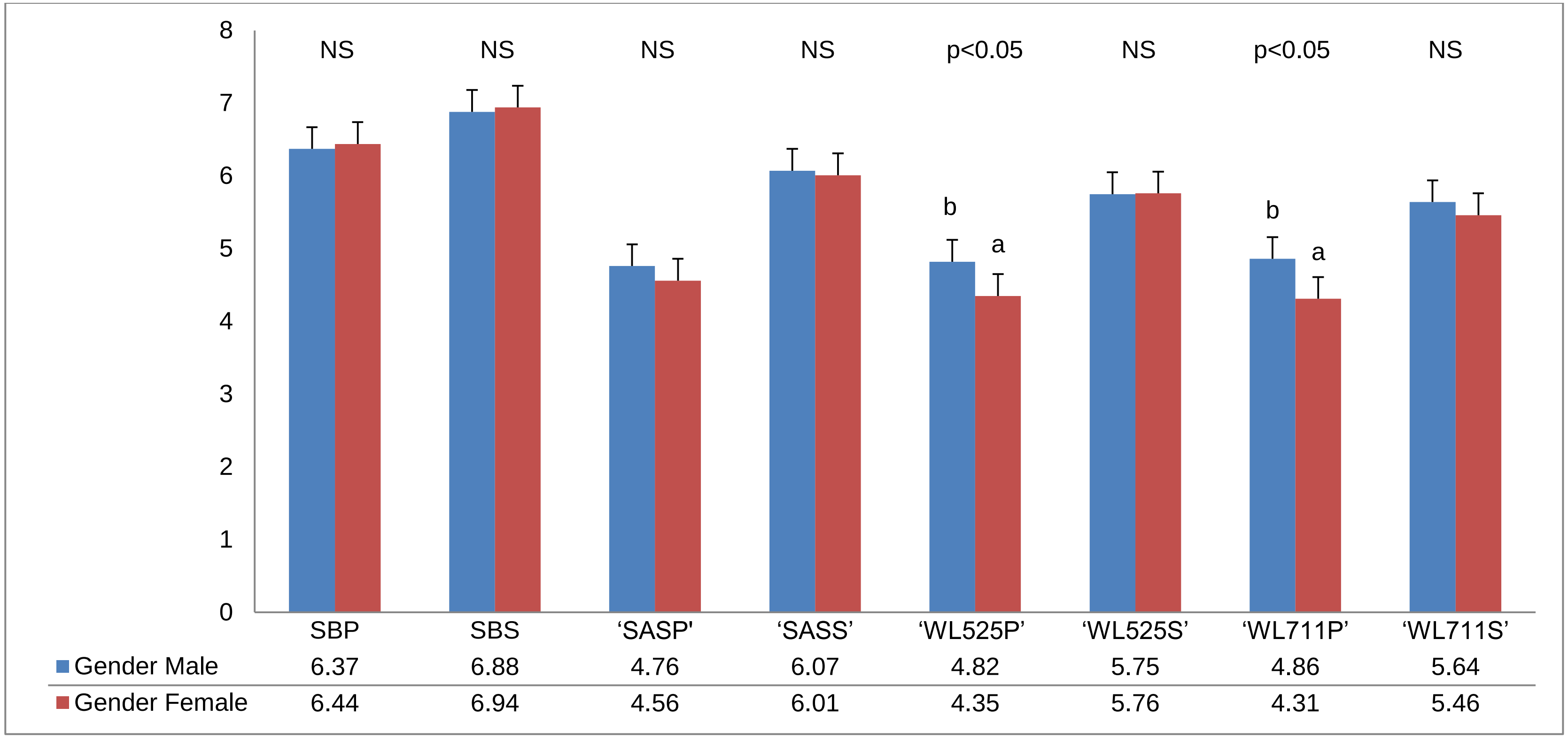
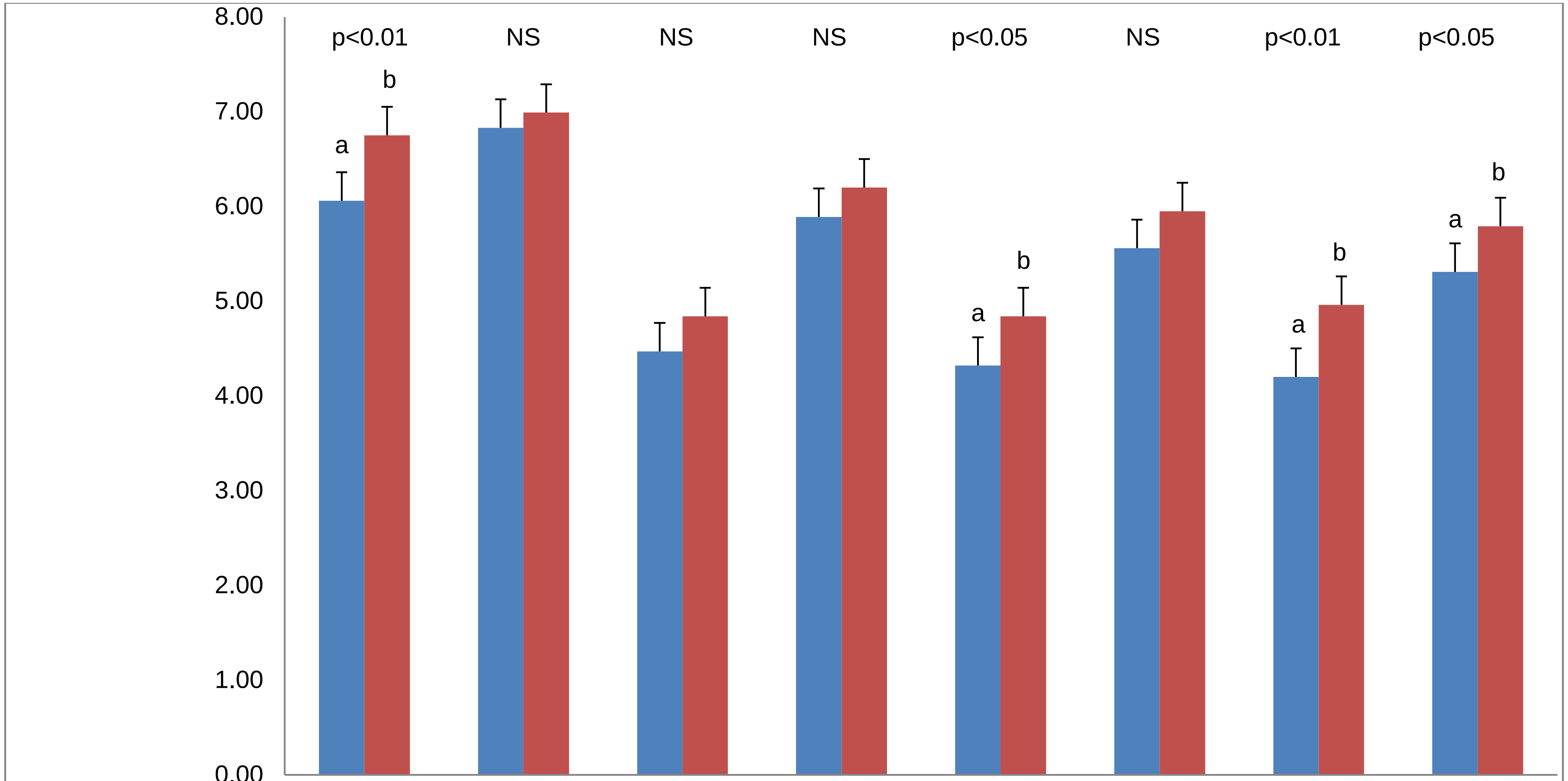


Fig. 4.6. Effect of gender on overall acceptability rating of eight samples.

Results with different superscripts differ significantly.

NS = Not significant; SBP = spinach beet plain; SBS = spinach beet stew; 'SASP' = 'SA Standard plain'; 'SASS' = 'SA Standard stew'; 'WL525P' = 'WL525 plain'; 'WL525S' = 'WL525 stew'; 'WL711P' = 'WL711 plain'; 'WL711S' = 'WL711 stew'

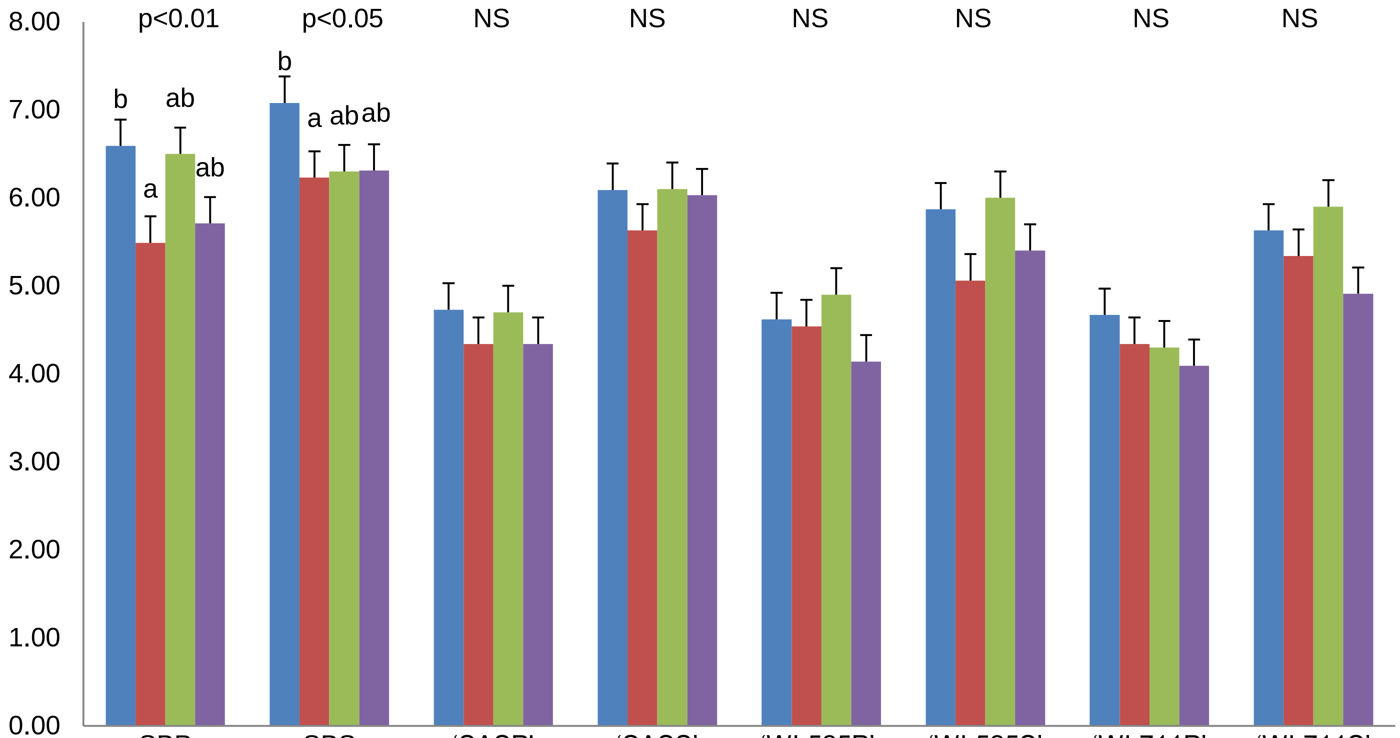


	SBP	SBS	'SASP'	'SASS'	'WL525P'	'WL525S'	'WL711P'	'WL711S'
Age 18 – 32 years	6.06	6.83	4.47	5.89	4.32	5.56	4.20	5.31
Age 33 – 65 years	6.75	6.99	4.84	6.20	4.84	5.95	4.96	5.79

Fig. 4.7. Effect of age on overall acceptability rating of eight samples.

Results with different superscripts differ significantly.

NS = Not significant; SBP = spinach beet plain; SBS = spinach beet stew; 'SASP' = 'SA Standard plain'; 'SASS' = 'SA Standard stew'; 'WL525P' = 'WL525 plain'; 'WL525S' = 'WL525 stew'; 'WL711P' = 'WL711 plain'; 'WL711S' = 'WL711 stew'



	SBP	SBS	'SASP'	'SASS'	'WL525P'	'WL525S'	'WL711P'	'WL711S'
Race Black African	6.59	7.08	4.73	6.09	4.62	5.87	4.67	5.63
Race Coloured	5.49	6.23	4.34	5.63	4.54	5.06	4.34	5.34
Race Indian/Asian	6.50	6.30	4.70	6.10	4.90	6.00	4.30	5.90
Race White	5.71	6.31	4.34	6.03	4.14	5.40	4.09	4.91

Fig. 4.8. Effect of race on overall acceptability rating of eight samples.

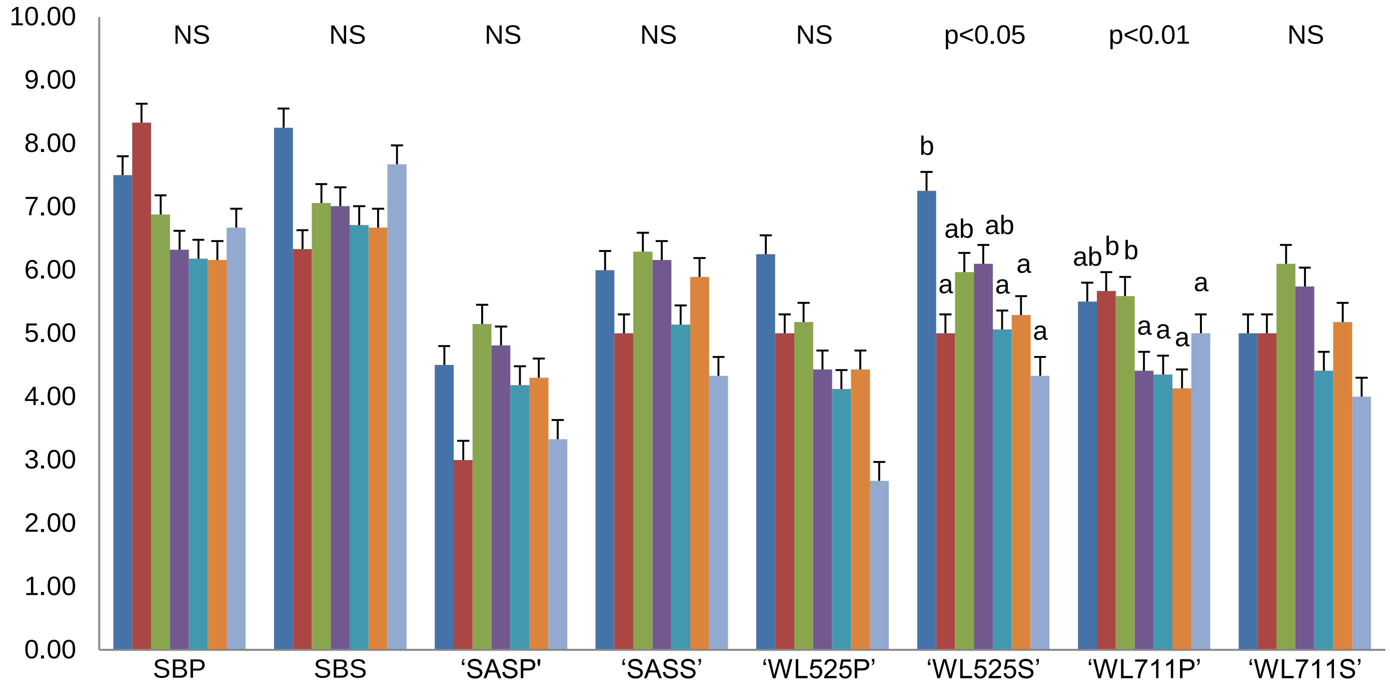
Results with different superscripts differ significantly.

NS = Not significant; SBP = spinach beet plain; SBS = spinach beet stew; 'SASP' = 'SA Standard plain'; 'SASS' = 'SA Standard stew'; 'WL525P' = 'WL525 plain'; 'WL525S' = 'WL525 stew'; 'WL711P' = 'WL711 plain'; 'WL711S' = 'WL711 stew'

Education (Figure 4.9), as main effect, showed two significant differences. Consumers with ‘some primary’ education (0.78; Table 4.1) indicated that ‘WL525S’ (7.25) was significantly ($p<0.05$) more acceptable to them, than consumers with a ‘primary completed’ (5.00), ‘technical diploma/degree’ (5.06), ‘university degree’ (5.29) and ‘other’ level of education (4.33) (Figure 4.9). The higher acceptance towards ‘WL525S’ could be because of the high Brix (sucrose content) value of 18.90%, that was determined in Chapter 3 (section 3.3.2; Table 3.2), indicating that ‘WL525S’ was sweeter in taste. Furthermore, the trained panel also found (section 4.3.1) that ‘WL525S’ was the least grassy in aroma (1.41) (section 4.3.1.1) and high in onion aroma (4.22) (section 4.3.1.3). Consumers from all education levels indicated that SBP was more acceptable, although not significant, than all the plain and stew samples (Figure 4.9).

Consumers with a ‘primary completed’ (5.67) and ‘some high’ (5.59) education, indicated that ‘WL711P’ was significantly ($p<0.01$) more acceptable to them than consumers with a ‘matric (grade 12)’ (4.41), ‘technicon diploma/degree’ (4.35) and ‘university degree’ (4.13) (Figure 4.8). These differences can be explained by studies that have indicated that the level of education can influence dietary behaviour during adulthood (Kearney, Kearney, Dunne, & Gibney, 2000). Brewer and Novakofsky (2008) also identified education as one of three aspects of the buyer’s profile that contributed most to variation in sensory attribute ratings. As consumers have a low level of education and because they are exposed less to more sophisticated foods, they only eat foods that they are familiar with. Furthermore, Hjelmar (2011) and Ree, Riediger and Moghadasian (2007) confirmed that level of education and income influenced food choice via the availability of the resources to purchase a higher quality food and awareness of nutritious alternatives.

However, in the present study, income (Figure 4.10), as main effect, showed no significant differences. This could be explained by the fact that consumers are still unfamiliar with lucerne as a food source. Therefore, consumers have less information in their memory to guide them to make an informed choice. A consumers’ diet may vary depending on the availability of income to purchase healthier, nutrient-rich foods (Hjelmar, 2011). For low-income consumers, the price of food products is more important than the taste and quality. This may partly explain the lower life expectancy of lower-income consumers (Steenhuis, Waterlander, & De Mul, 2011). It is therefore evident that different factors are important for consumers with different levels of income.

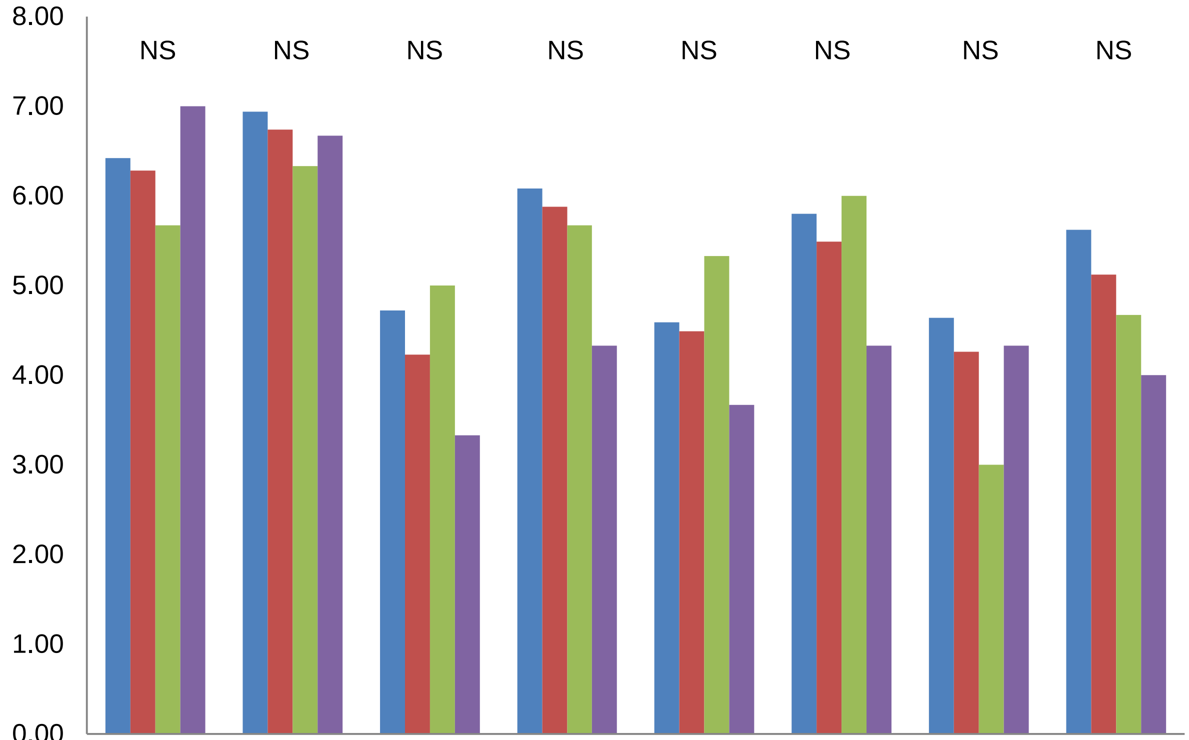


	SBP	SBS	'SASP'	'SASS'	'WL525P'	'WL525S'	'WL711P'	'WL711S'
Some primary	7.50	8.25	4.50	6.00	6.25	7.25	5.50	5.00
Primary completed	8.33	6.33	3.00	5.00	5.00	5.00	5.67	5.00
Some High school	6.88	7.06	5.15	6.29	5.18	5.97	5.59	6.10
Matric (Grade 12)	6.32	7.01	4.81	6.16	4.43	6.10	4.41	5.74
Tech diploma/degree	6.18	6.71	4.18	5.14	4.12	5.06	4.35	4.41
University degree	6.16	6.67	4.30	5.89	4.43	5.29	4.13	5.18
Other	6.67	7.67	3.33	4.33	2.67	4.33	5.00	4.00

Fig. 4.9. Effect of education on overall acceptability rating of eight samples.

Results with different superscripts differ significantly.

NS = Not significant; SBP = spinach beet plain; SBS = spinach beet stew; 'SASP' = 'SA Standard plain'; 'SASS' = 'SA Standard stew'; 'WL525P' = 'WL525 plain'; 'WL525S' = 'WL525 stew'; 'WL711P' = 'WL711 plain'; 'WL711S' = 'WL711 stew'



	SBP	SBS	'SASP'	'SASS'	'WL525P'	'WL525S'	'WL711P'	'WL711S'
Income per year R0 – R100 000	6.42	6.94	4.72	6.08	4.59	5.80	4.64	5.62
Income per year R101 – R500 000	6.28	6.74	4.23	5.88	4.49	5.49	4.26	5.12
Income per year R501 – R750 000	5.67	6.33	5.00	5.67	5.33	6.00	3.00	4.67
Income per year More than R750 000	7.00	6.67	3.33	4.33	3.67	4.33	4.33	4.00

Fig. 4.10. Effect of income on overall acceptability rating of eight samples.

Results with different superscripts differ significantly.

NS = Not significant; SBP = spinach beet plain; SBS = spinach beet stew; 'SASP' = 'SA Standard plain'; 'SASS' = 'SA Standard stew'; 'WL525P' = 'WL525 plain'; 'WL525S' = 'WL525 stew'; 'WL711P' = 'WL711 plain'; 'WL711S' = 'WL711 stew'

Figure 4.11 shows the frequency of the hedonic scale ratings for overall acceptability of the eight tasted samples. For the positive frequency, ‘moderately liked’ was the positive reaction mostly chosen by the respondents. Respondents ‘moderately liked’ SBP (*n* = 108), SBS (*n* = 116), ‘SASP’ (*n* = 76), ‘SASS’ (*n* = 92), ‘WL525S’ (*n* = 110), ‘WL711P’ (*n* = 75) and ‘WL711S’ (*n* = 84). For the negative frequency, ‘slightly disliked’ was the negative reaction mostly chosen by the respondents. Respondents ‘slightly disliked’ SBP (*n* = 19), SBS (*n* = 13), ‘SASP’ (*n* = 46), ‘SASS’ (*n* = 22), ‘WL525S’ (*n* = 39), ‘WL711P’ (*n* = 46) and ‘WL711S’ (*n* = 38).

From the above results, it can be summarized that ‘SASS’ was the most preferred lucerne cultivar. The numerical values for degree of liking for aroma, mouthfeel, taste and overall acceptability for ‘SASS’ were higher than for ‘WL525’ and ‘WL711’ (Figure 4.4). The preference for ‘SASS’ was also higher than ‘WL525’ and ‘WL711’. Consumers also indicated that they were more likely to purchase ‘SASS’ within two weeks, one month, in supermarkets, use it as a substitute for SB and get used to the taste of it (Figure 4.5). Not only was the average gain in the degree of liking for taste, mouthfeel and overall acceptability for ‘SAS’ higher than the other two lucerne cultivars (Table 4.5), but also the average gain to purchase the tested sample within two weeks, one month and in a supermarket and to get used to the taste of the tested sample.

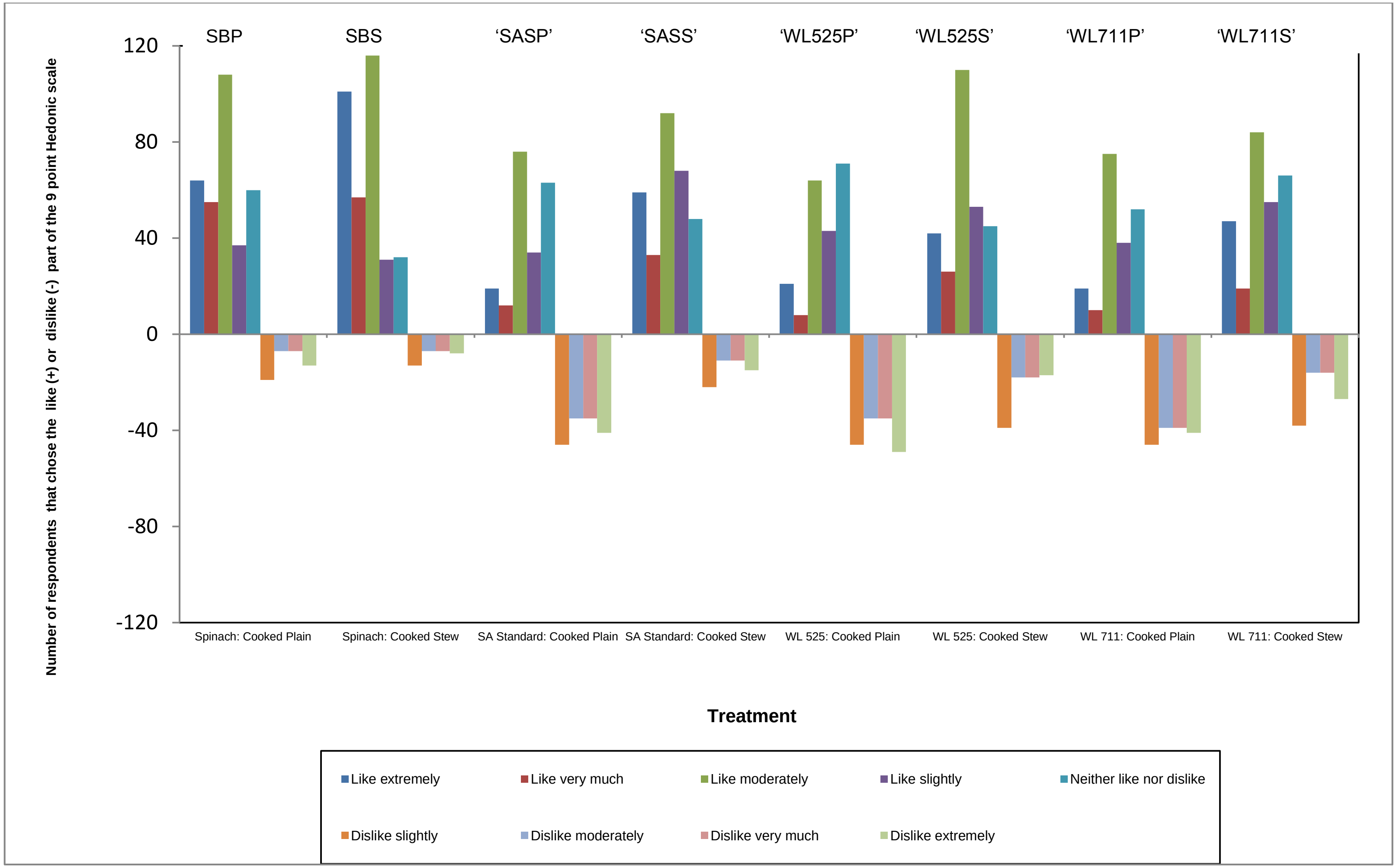


Fig. 4.11. The frequency of hedonic scale ratings for overall acceptability per product.

4.4 Conclusions

The potential of lucerne for human consumption has increased among consumers, because of its high nutritive value. The sensory attributes of different lucerne cultivars, by means of sensory DA, as well as consumers' acceptance towards lucerne, has yet not been determined in SA.

Panellists generated and reached consensus for 13 (plain samples) and 15 (stew samples) sensory descriptors. Of all the lucerne samples described, 'SASS' showed the lowest values for fibrous appearance, chewy and fibrous mouthfeel, bitter taste, and bitter and metallic aftertaste.

From the PCA biplot, three stew samples (SB, 'SAS' and 'WL525') and the SBP were described by the more 'positive' attributes, such as soft and wet appearance, spinach beet aroma and salty taste. 'WL711 stew' and three plain samples ('SAS', 'WL525' and 'WL711') were associated with the more 'negative descriptors, namely grassy aroma, fibrous and chewy texture, fibrous appearance, bitter taste, and bitter and metallic aftertaste.

As the taste and mouthfeel of different cultivars may contribute to the taste acceptability of green leafy vegetables, such as SB and lucerne, new emerging cultivars can be profiled using the lexicon that was developed in this study, to identify sensory differences among different lucerne cultivars. In addition, these findings have important implications for overall consumer acceptability of the different lucerne cultivars and can be used in future studies to profile new emerging cultivars.

With the acceptance test, major differences were found for the degree of liking for taste and texture, while degree of liking for aroma, overall acceptability and preference were more closely contested. The SBS was the most preferred sample, followed by SBP and 'SASS' (Figure 4.4). It was argued that a possible explanation for the lower acceptance towards lucerne could be due to behavioural hesitation to eat food that is originally used as animal feed in SA. Lucerne cultivar 'SAS' indicated the highest acceptability and preference among the lucerne cultivars.

The average gain for consumers' acceptance, preference and purchase intention between the lucerne plain and stew samples indicated that, while the level of acceptance for both SBP and SBS were the same, the plain lucerne samples improved in acceptance with the addition of potato and onion. Therefore, the method of preparation and addition of ingredients played an important role to make the lucerne samples more acceptable for consumers.

CHAPTER 5

THE RELATIONSHIP BETWEEN CONSUMER ACCEPTABILITY AND DESCRIPTIVE SENSORY ATTRIBUTES OF LUCERNE (*Medicago sativa* L.), BY USING PREFERENCE MAPPING

ABSTRACT

The objective of this study was to determine the relationship between consumer acceptability and descriptive sensory attributes of three lucerne cultivars and one spinach beet cultivar, by using external preference mapping. The results indicated that three consumer clusters, with different lucerne preferences, were obtained by agglomerate hierarchical clustering. Further results indicated that differences in consumer preference occurred when the plain and stew samples were analysed separately, and then analysed in combination. Although all the consumer clusters preferred the plain and stew spinach beet samples, 'SA Standard stew' was the lucerne sample preferred by all the consumers, except those with some primary education and a yearly income of R501 000 – R750 000 per year, who indicated only a high acceptance for this sample.

5.1 Introduction

Preference mapping (PM) is a method to investigate consumer preference for a series of products, by using statistical mapping methods (e.g. PCA and principle component regression). Consumer preference data can be analysed, without taking other data sets into account. However, the main interest usually lies in understanding the relationship between consumer preference and sensory attributes of the products. The procedure requires objective characterization of a product's sensory attributes, by e.g. GDA. This data is then related to preference ratings for the product, obtained from a representative sample of consumers (Murray & Delahunty, 2000). According to Helgesen and Næs (1995), important application areas for PM are product development and product improvement. Preference mapping has also been used to study the acceptability of food products such as meat, beverages, fruits and cheese (Sveinsdóttir et al., 2009).

Preference mapping can broadly be categorised into internal and external PM. Internal PM uses consumer acceptance ratings to locate the products on the maps. External PM, which was used in this study, uses sensory descriptive attribute ratings to locate the products on the maps (Caspia et al., 2006; McEwan, 1996; Meilgaard et al., 2007). Also, external PM aims at explaining the sensorial source of consumers' preferences and is a more informative tool than internal PM. Numerous studies have used external PM to analyse consumers' acceptability of various products, such as fermented sausages, apples, honey, cheese, tomato and other products (Gámbaro, Ares, Giménez, & Pahor, 2007; Jaeger, Andani, Wakeling, & MacFie, 1998; Lawlor & Delahunty, 2000; Lé & Ledauphin, 2006; Martins-Medeiros-De-Melo, André-Bolini, & Efraim, 2009; Resano, Sanjuán, & Albisu, 2007; Rødbotten et al., 2009).

The aim of this chapter was to determine how the descriptive attributes for the three lucerne cultivars and one SB cultivar related to consumer acceptability, by using external PM. As the relationship between consumer acceptability and descriptive sensory attributes of lucerne cultivars have never been studied in SA, it was necessary to perform PM on all three lucerne cultivars, in order to obtain an objective characterization of lucerne's sensory attributes. Furthermore, the results obtained from all the lucerne cultivars will assist in the development and improvement of an acceptable lucerne product for SA consumers.

5.2 Materials and methods

5.2.1 Preparation of samples

Three lucerne cultivars ('SA Standard', 'WL711' and 'WL525') and one SB cultivar were prepared, as described in section 4.2.1 of Chapter 4, for the consumer acceptance and GDA tests.

5.2.2 Consumer panel

A consumer panel (*n* = 384) evaluated the lucerne and SB samples for acceptability, at the sensory laboratory of the Food Science Division, Department of Microbial, Biochemical and Food Biotechnology, UFS (sections 4.2.5 – 4.2.6 in Chapter 4).

5.2.3 Generic descriptive analysis

Generic descriptive analysis was performed by a trained and experienced panel (*n* = 9) (sections 4.2.2 – 4.2.4 in Chapter 4). Attributes and definitions for the plain and stew samples were presented in Tables 4.1 and 4.2, respectively, in Chapter 4.

5.2.4 Statistical analysis

XLSTAT 2007 was used to analyse data from the consumer panel. The significance of the overall acceptance, measured for each sample, was tested by means of ANOVA (NCSS, 2007). The different samples were used as the main effects at a significance level of 95 % ($p \leq 0.05$). If the main effect was significant, Fisher's LSD-test was applied to determine the direction of the differences between mean values. The descriptive data was analysed by PCA, using XLSTAT (2007), to examine the degree of the panellists' agreement (Dijksterhuis & Punter, 1990). Agglomerate hierarchical clustering (AHC) was performed to group consumers, based on their acceptance of lucerne. External PM was conducted on the acceptability scores of the consumer panel, to determine the relationship between sensory attributes and consumer preference.

5.3 Results and discussion

In Chapter 4, consumers' acceptance and sensory descriptive attributes of three lucerne cultivars were determined. It was found that lucerne cultivar 'SAS' indicated the highest acceptability and was the most preferred lucerne cultivar. 'SA Standard stew' also had the highest values, of all the lucerne samples, for spinach aroma, soft and wet appearance, mushy appearance and mouthfeel, and salty taste. On the other hand, 'SASS' indicated the lowest value of all the samples for potato aroma. When comparing the lucerne samples, 'SASS' had the lowest value for: fibrous appearance; chewy and fibrous mouthfeel; bitter taste; and bitter and metallic aftertaste. Therefore, external PM was performed on both the plain and stew samples, separately, as well as on the combined samples, to relate descriptive analysis data with consumer acceptability.

Three clusters of consumers, with different lucerne and SB preferences, were obtained by AHC (Figures 5.1, 5.4 & 5.7). A dendrogram represented how the algorithm grouped the observations, with the dotted line signifying the automatic truncation, resulting in three groups (XLSTAT, 2007). The dendrogram for the plain samples, stew samples, and the plain and stew samples combined, were truncated at the 126, 161 and 254 levels, respectively, which were the cut-off points allocated by the statistical program. The vertical axis of the dendrogram represented the distance or dissimilarity between clusters and the horizontal axis, the objects and clusters. Each joining (fusion) of two clusters was represented on the graph by the splitting of a vertical line into two vertical sub-lines. The vertical position of the split, shown by the short horizontal bar, gave the distance (dissimilarity) between the two clusters (NCSS, 2007). These dendrograms indicated the most preferred sample. To explain the consumer clustering and the attributes of the samples, the PCA biplots were again included.

When evaluating the results of the plain samples (Figure 5.1), cluster 1 linked near the left side of the plot, indicating that cluster 1 was different from the other two clusters. Clusters 2 and 3 linked near the right side of the plot, indicating little difference. From the PM

(Figure 5.2), it was clear that all three consumer groups preferred the SBP sample (Figure 5.3).

In Chapter 4 (Figure 4.11) the frequency of the hedonic scale ratings for overall acceptability indicated that 108 consumers liked SB. For cluster 1, 39.2% of the panellists preferred plain samples that were softer in mouthfeel and appearance (Figure 5.3). For cluster 2, 24.3% of the panellists preferred a wet appearance and salty taste, while for cluster 3, 36.4 % of the panellists preferred grassy and spinach aroma. These findings could be linked to the data in Chapter 4 (Table 4.4), as SBP was not only more grassy and spinach in aroma than all three plain lucerne cultivars, but also softer and wetter in appearance, and softer in mouthfeel.

When looking at the effect of consumer profiles on the hedonic ratings in Chapter 4 (section 4.3.4), the three clusters comprised of consumers of the female gender (Figure 4.6), 33 – 65 age group (Figure 4.7), Indian/Asian race group (Figure 4.8), primary completed education level group (Figure 4.9) and > R750 000 income level group (Figure 4.10). The three clusters are not a composite profile, but rather consist of individual demographical characteristics. Means for this group ranged from 6.44 to 8.33 (Chapter 4, section 4.3.3) out of a nine-point hedonic scale, indicating the overall acceptability for SBP.

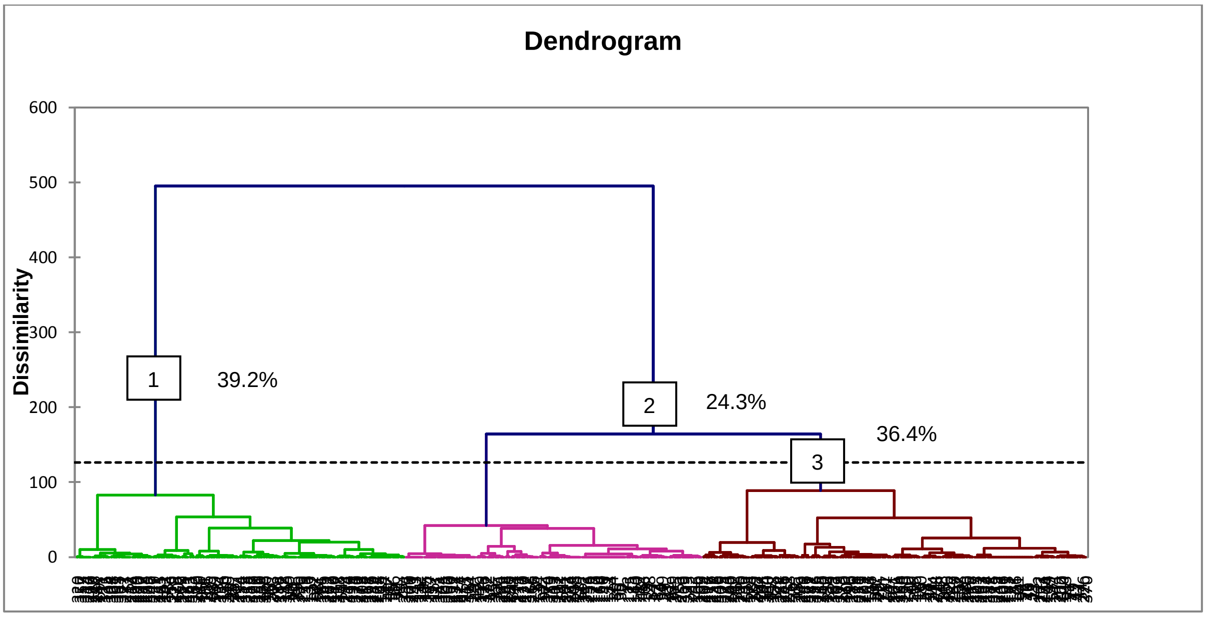


Fig. 5.1. Dendrogram of the three major consumer clusters for the plain lucerne and spinach beet samples.

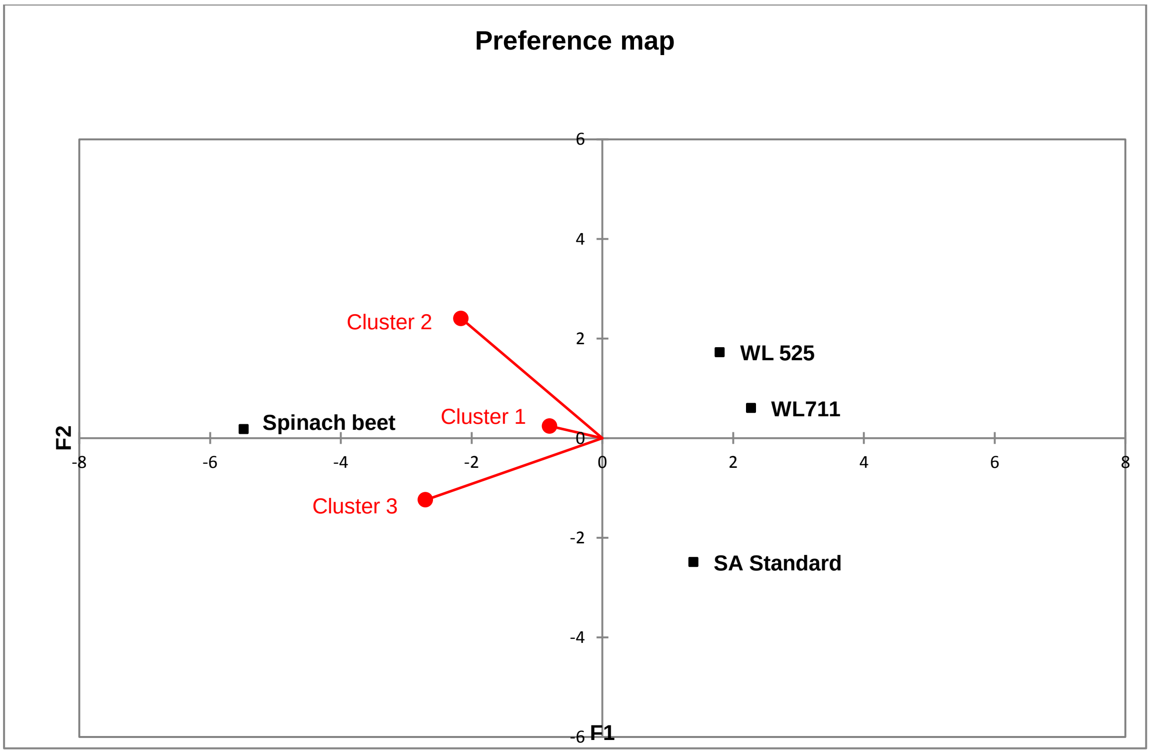


Fig. 5.2. External preference map of the three consumer clusters for the plain lucerne and spinach beet samples.

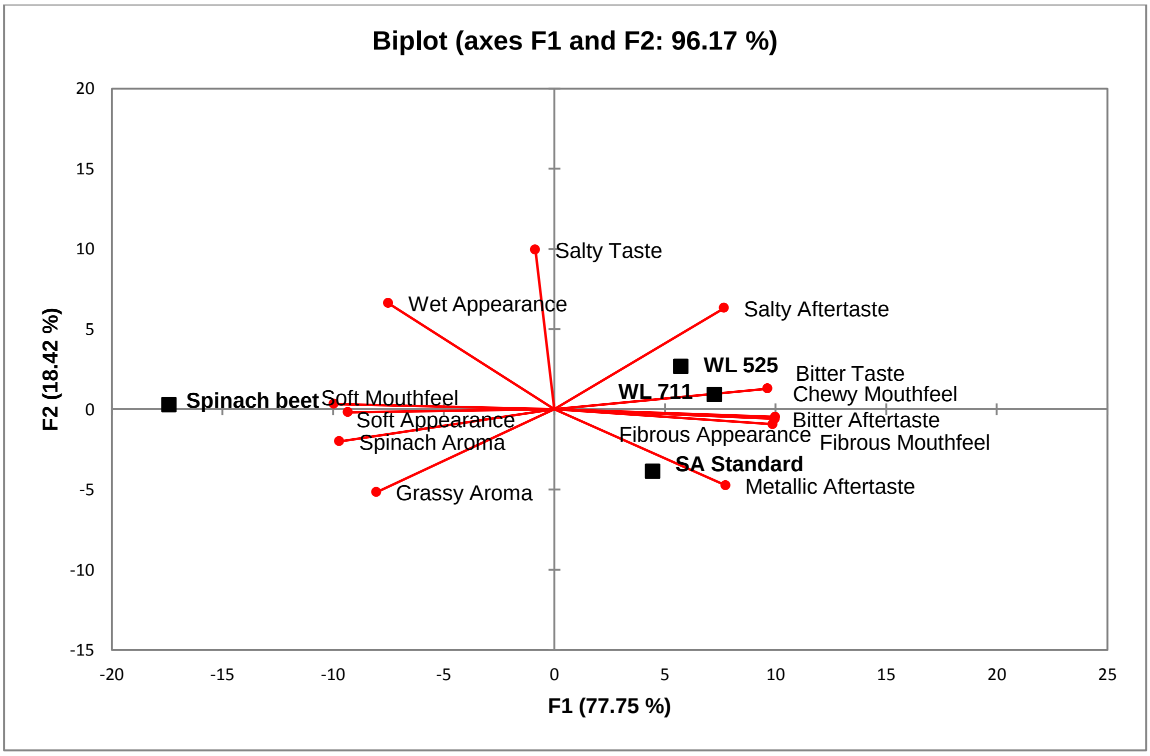


Fig. 5.3. Principal Component Analysis biplot of the plain lucerne and spinach beet samples.

From the evaluation of the stew samples (Figure 5.4), cluster 1 linked near the left side of the plot, indicating that cluster 1 was different from the other two clusters. Clusters 2 and 3 were linked near the right side of the plot, again indicating little dissimilarity.

The PM (Figure 5.5) and PCA (Figure 5.6) biplots for the stew lucerne and SB samples again indicated that all three consumer clusters preferred the stew SB sample. In Chapter 4 (Figure 4.11), the frequency of the hedonic scale ratings for overall acceptability indicated that 116 consumers liked SBS. For cluster 1, 19.9% of the panellists, preferred the stew SB sample with potato and onion aroma, and soft appearance. For cluster 2, 11.4% of the panellists, preferred a mushy and wet appearance and spinach aroma, while for cluster 3, 66.2% of the panellists preferred a salty taste (Figures 5.5 & 5.6). These findings could be linked to the data in Chapter 4 (Table 4.4), as SBS was not only more spinach, onion and potato in aroma than all three plain lucerne cultivars, but also softer, mushier and wetter in appearance, and more salty in taste. The closest lucerne cultivar to these attributes was 'SASS' (Figures 5.5 & 5.6).

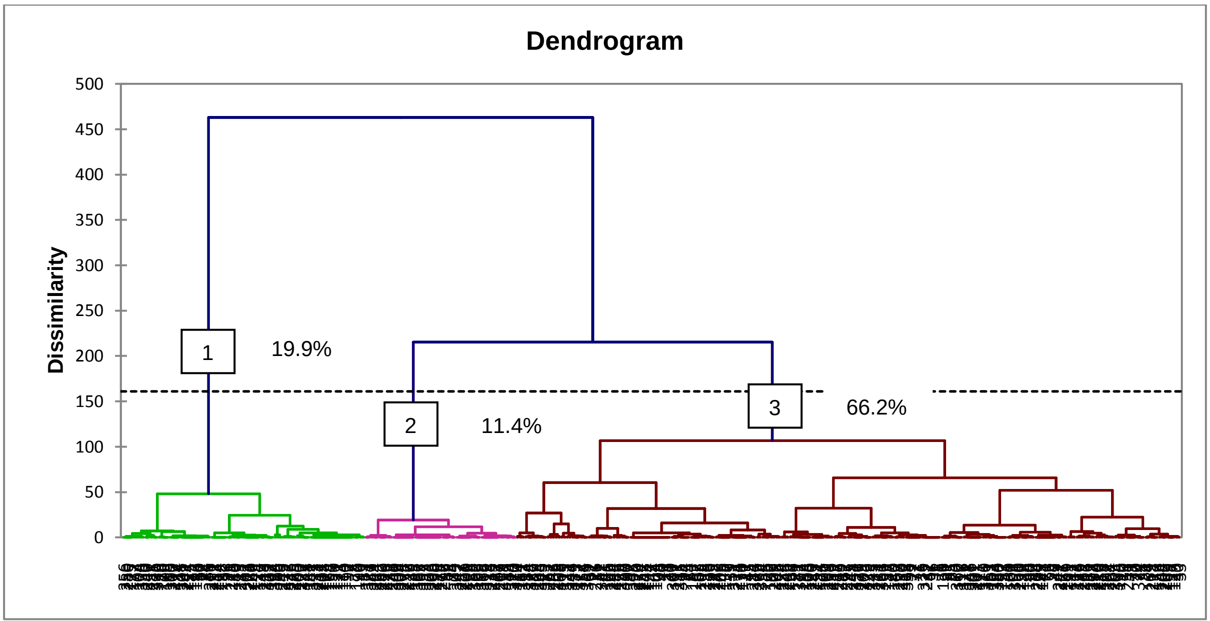


Fig. 5.4. Dendrogram of the three major consumer clusters for the stew lucerne and spinach beet samples.

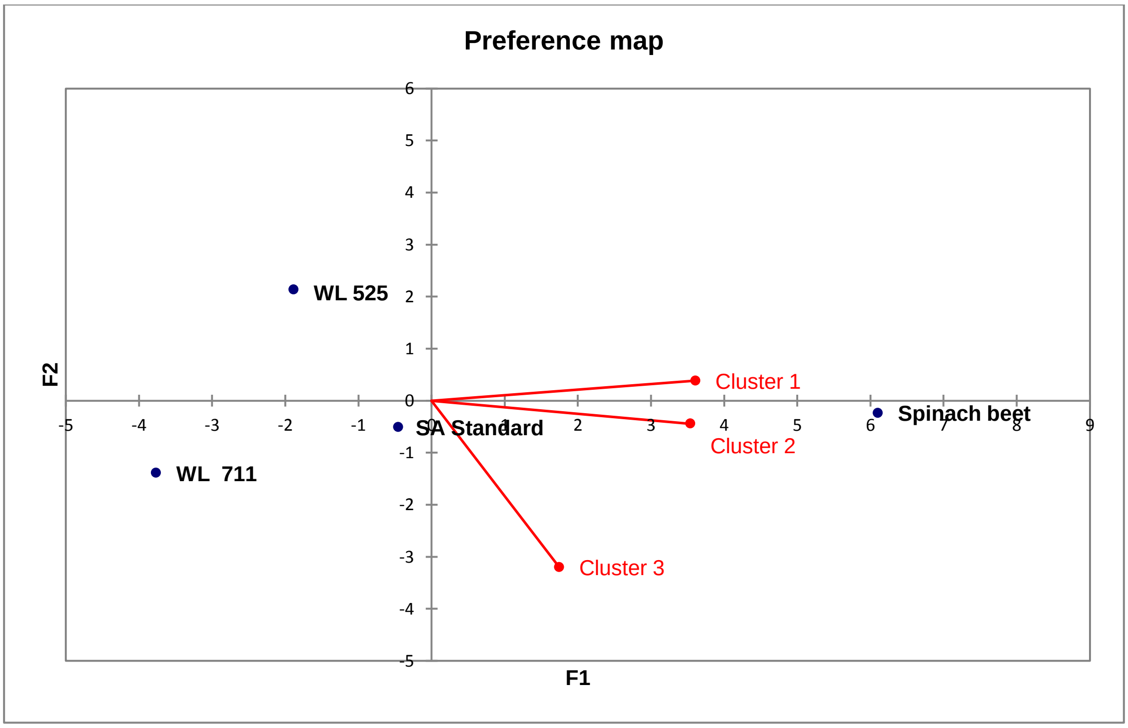


Fig. 5.5. External preference map of the three consumer clusters for the stew lucerne and spinach beet samples.

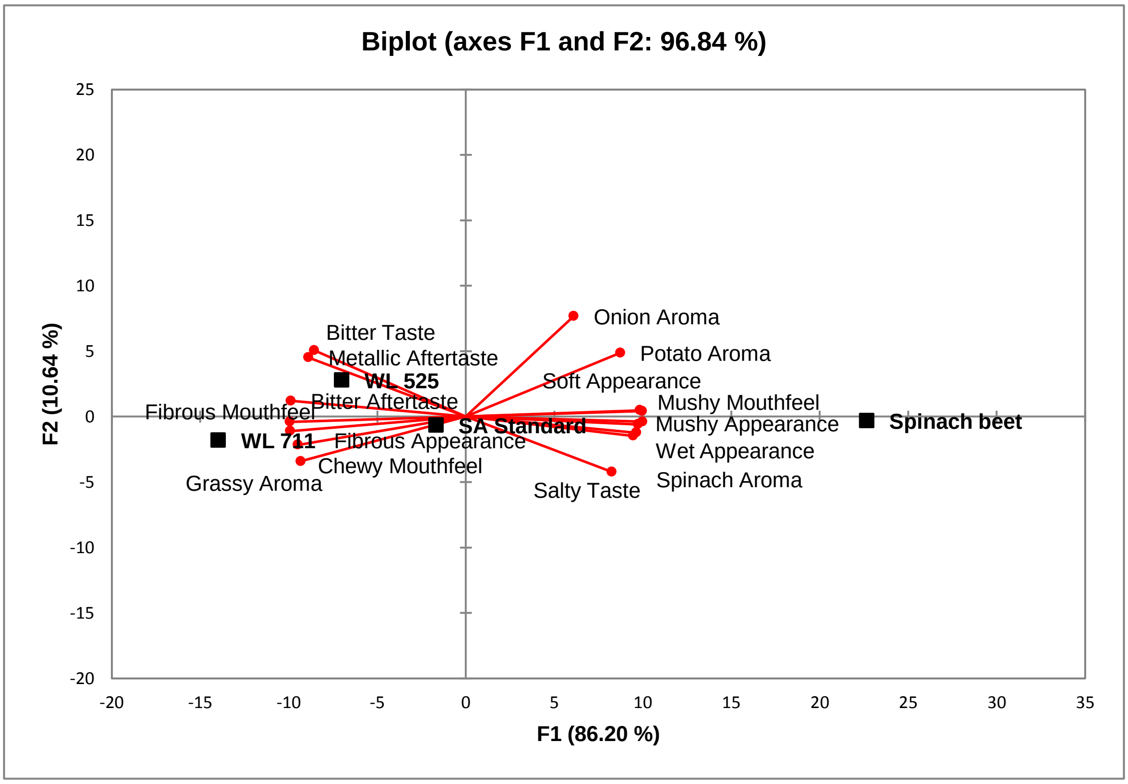


Fig. 5.6. Principal Component Analysis biplot of the stew lucerne and spinach beet samples.

The effect of consumer profiles on the hedonic ratings in Chapter 4 (section 4.3.4), indicated that the three clusters comprised of females (Figure 4.6), 33 – 65 years old (Figure 4.7), Black Africans (Figure 4.8), with some primary education (Figure 4.9) and earning R0 – R100 000 per year (Figure 4.10); these consumers had the highest acceptance for SBS, as their means ranged from 6.94 to 8.25 (Chapter 4, section 4.3.3) out of a nine-point hedonic scale, indicating the overall acceptability for SBS. All the consumers, except those with a primary completed education and who earned > R750 000 per year, indicated a higher acceptance for SBS than all the samples. Therefore, it could be speculated that these consumers fall into these three clusters.

From the results of the combined samples (Figure 5.7), the same tendency as for the stew dendrogram could be noted. Again cluster 1 linked near the left side of the plot, indicating that this cluster was different from the other two clusters, which were linked near the right side of the plot, indicating little variation.

The PM (Figure 5.8) and PCA (Figure 5.9) biplots for all the samples showed that all three consumer clusters preferred SBP, SBS and 'SASS'. In Chapter 4 (Figure 4.11), the frequency of the hedonic scale ratings for overall acceptability indicated that 92 consumers liked SBS. For cluster 1, 21.5% of the panellists preferred stew samples with a soft appearance and spinach aroma. The 'WL525S' could be characterised by these attributes. For cluster 2, 45.4% of the panellists preferred the stew samples with a wet appearance, salty taste and spinach aroma (Figures 5.8 & 5.9). Spinach beet stew and 'SASS' could be characterised by these attributes. For cluster 3, 33.1% of the panellists preferred plain samples with a soft appearance and grassy aroma. Spinach beet plain could be characterised by these attributes.

The effect of consumer profiles on the hedonic ratings in Chapter 4 (section 4.3.4), indicated that males (Figure 4.6), 33 – 65 years old (Figure 4.7), Indians/Asians (Figure 4.8), with some high school education (Figure 4.9) and earning R0 – R100 000 per year (Figure 4.10) showed the highest acceptance for 'SASS'. Means for this group ranged from 6.07 to 6.29 (Chapter 4, section 4.3.3) out of a nine-point hedonic scale, indicating the overall acceptability for 'SASS'. Also, all the consumers, except those with some primary education (Figure 4.9) and who earned between R501 000 – R750 000 per year (Figure 4.10), indicated a high acceptance for 'SASS'. Therefore, it could be speculated that this profile of consumers fall into cluster 2.

Female consumers (Figure 4.6), age 33 – 65 years (Figure 4.7), Black Africans (Figure 4.8), with some primary education (Figure 4.9) and earning R0 – R100 000 per year (Figure 4.10) indicated the highest acceptance for SBS. Means for this group ranged from 6.94 to 8.25 (Chapter 4, section 4.3.3) out of a nine-point hedonic scale, indicating the overall acceptability for SBS. Therefore, it can be speculated that these consumers fall into cluster 2.

A high acceptance for 'WL525S' was shown by females (Figure 4.6), age 33 – 65 years (Figure 4.7), Indians/Asians (Figure 4.8), with some primary education (Figure 4.9) and earning between R501 000 – R750 000 per year (Figure 4.10). Means for this group ranged from 5.67 to 6.20 (Chapter 4, section 4.3.3) out of a nine-point hedonic scale, indicating the overall acceptability for 'WL525S'. Therefore, it can be speculated that these consumers fall into cluster 1.

For cluster 3, consumers of the female gender (Figure 4.6), 33 – 65 age group (Figure 4.7), Indian/Asian race group (Figure 4.8), primary completed education level group (Figure 4.9) and > R750 000 income level group (Figure 4.10) indicated a high acceptance for SBP. Again, it should be emphasized that cluster 3 is not a composite profile, but rather consists of individual demographical characteristics. Means for this group ranged from 6.44 to 8.33 (Chapter 4, section 4.3.3), indicating a generally high degree of acceptance for SBP.

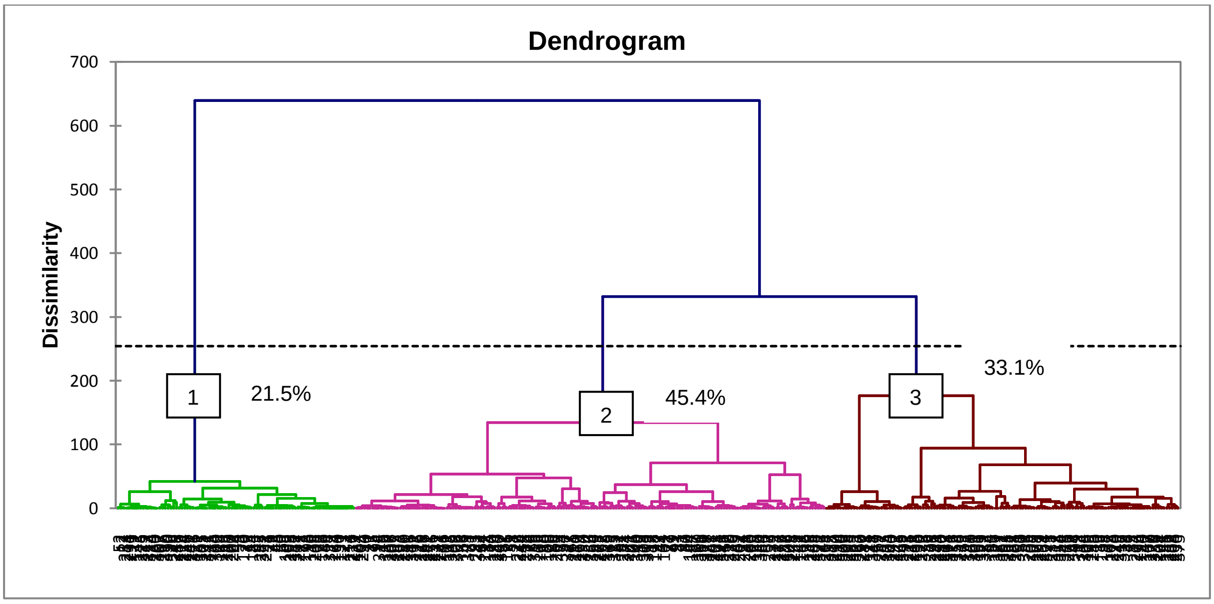


Fig. 5.7. Dendrogram of the three major consumer clusters for the plain and stew lucerne and spinach beet samples.

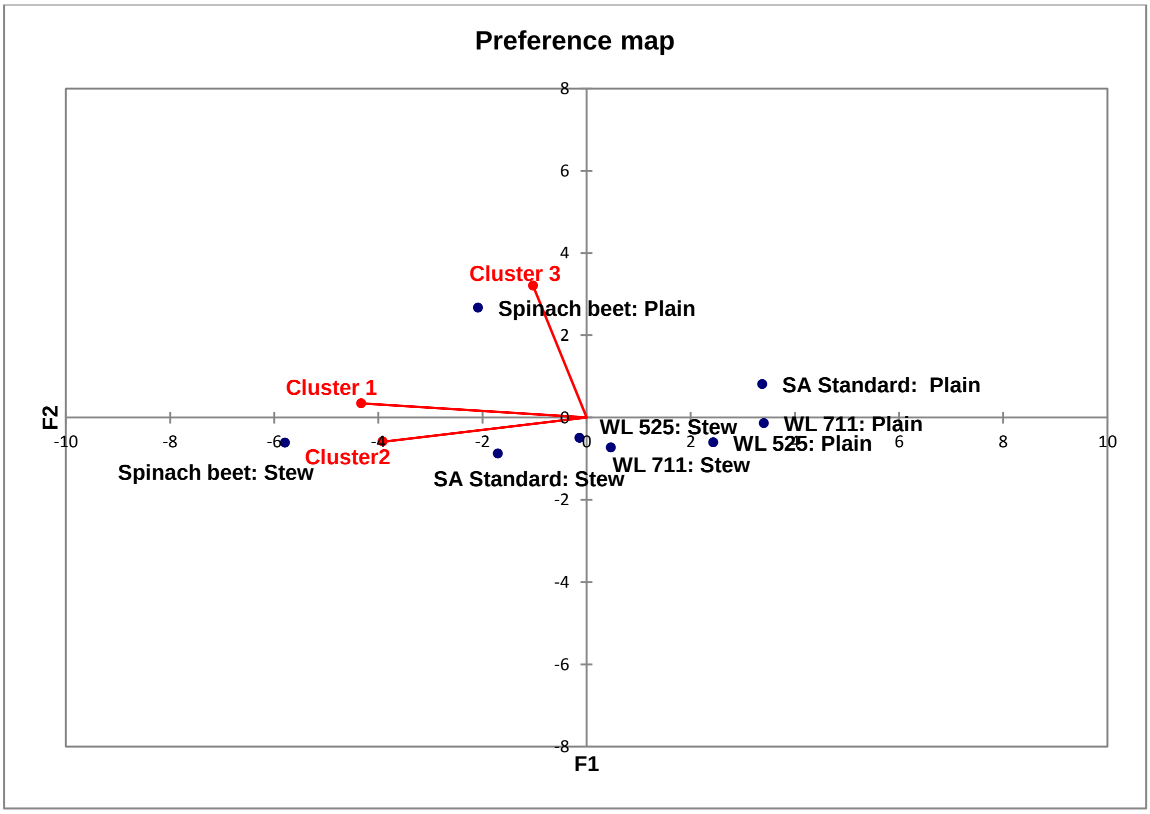


Fig. 5.8. External preference map of the three consumer clusters for the plain and stew samples.

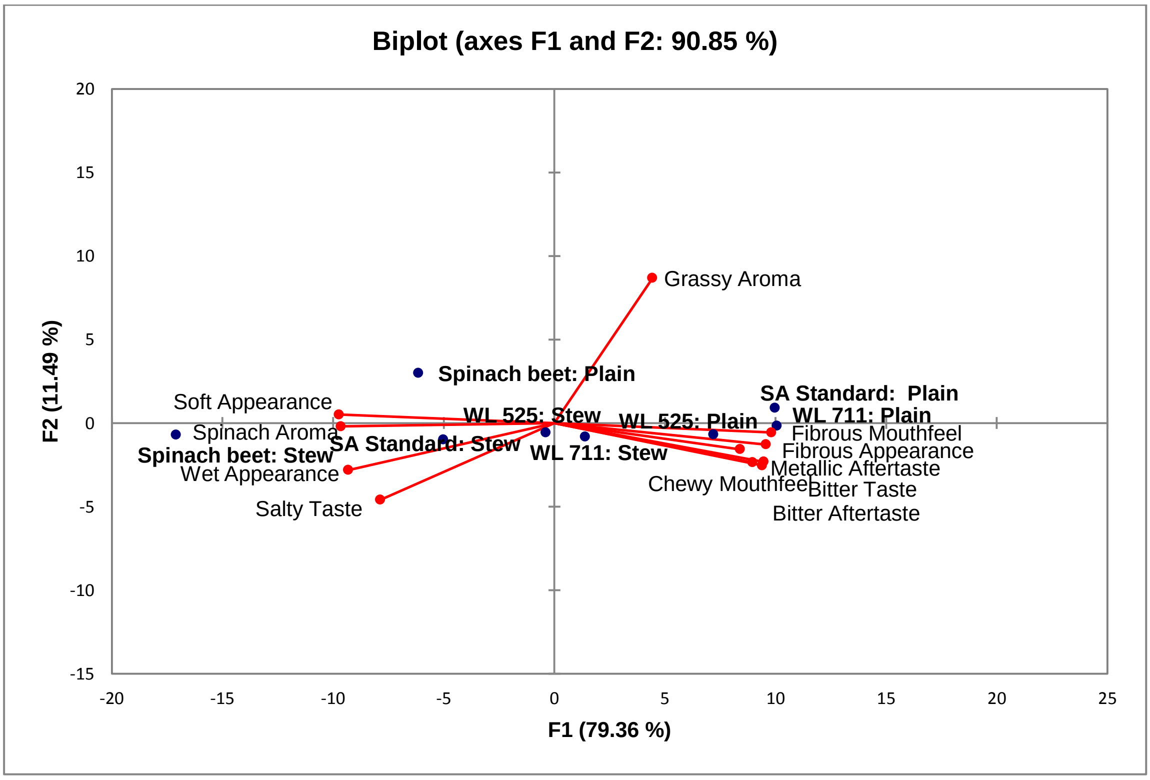


Fig. 5.9. Principal Component Analysis biplot of the stew lucerne and spinach beet samples.

According to Table 5.1, the lucerne sample that was preferred most by the consumers, was ‘SASS’, because of its wet appearance, salty taste and spinach aroma. Both SBP and SBS were preferred by all consumers. Since the consumers already knew the flavour of SB, they were familiar with it and could link it to the flavour of ‘SASS’. The fact that SB was also already part of the consumers’ diet, therefore creating an existing knowledge of it, also enforced the link with the ‘SASS’ (Peter & Olson, 2008).

Table 5.1

Summary of attributes of the plain, stew and combined (plain + stew) lucerne and spinach beet samples in the three clusters.

	Cluster 1	Cluster 2	Cluster 3	Cultivar(s)
Plain	Soft mouthfeel Soft appearance	Wet appearance Salty taste	Grassy aroma Spinach aroma	SBP
Stew	Potato aroma Onion aroma	Mushy appearance Wet appearance Spinach aroma	Salty taste	SBS
Combined (plain + stew)	Soft appearance Spinach aroma	Wet appearance Salty taste Spinach aroma	Soft appearance Spinach aroma	‘WL525S’; SBS; ‘SASS’; SBP

SBP = spinach beet plain; SBS = spinach beet stew; ‘SASS’ = ‘SA Standard stew’

5.4 Conclusions

External PM was applied to determine the relationship between consumer acceptability and descriptive sensory attributes of three lucerne cultivars one SB cultivar. Different results were obtained when the samples were analysed both separately and combined. With the plain samples, consumers in clusters 1 and 2 preferred SBP. With the stew samples, all three clusters preferred the SBS. With the combined results, consumers in cluster 1 preferred the ‘WL525S’. Clusters 2 preferred SBS and ‘SASS’, but cluster 3 preferred SBP. It was evident that ‘SASS’ was the preferred lucerne cultivar, as all the consumers, except those with some primary education and who earned between R501 000 – R750 000 per year, indicated a high acceptance for ‘SASS’. This sample was described as being wet in appearance, with a salty taste.

From the above results, and the results presented in Chapters 3 and 4, it could be concluded that lucerne cultivar ‘SAS’ was the most acceptable lucerne cultivar, to be used for product development and as a possible source of vegetable protein.

CHAPTER 6
CONSUMERS' KNOWLEDGE ABOUT AND ATTITUDE TOWARDS LUCERNE
(*Medicago sativa* **L.)**

ABSTRACT

The objectives of this study were not only to determine consumers' knowledge from different demographic groups, but also to investigate the role of specific variables as antecedents of consumers' attitude towards lucerne. Consumers' beliefs regarding the advantages, disadvantages and associations of eating lucerne were recorded. Data were collected from a purposive sample of consumers (*n* = 384) from Bloemfontein, Free State Province, on a three part self-completion questionnaire, consisting of knowledge, attitude and demographical questions. Consumers' knowledge of lucerne was analysed by means of descriptive statistics. For the determination of attitude, hypotheses were represented via the conceptual model, as to depict health benefits, food safety risks, sensory qualities and synonyms as forerunners of consumers' attitudes towards lucerne. Statistical measures, such as reliability and factor analysis, descriptive statistics, correlation analysis and multiple linear regressions were performed. Thematic analysis was used to categorise consumers' beliefs regarding lucerne and coded to quantitative data. While 63.1% of the respondents knew what lucerne was, 77% was unfamiliar with the term 'alfalfa' and 90.3% never consumed lucerne before. Younger respondents of the white population group, with a grade 12 or higher education, were significantly ($p<0.001$) more knowledgeable about lucerne. Further results indicated that there was a significant ($p<0.001$) relationship between consumers' attitude towards sensory qualities and synonyms, and their attitude towards lucerne. While respondents believed that 'health general' (40.1%) was the most important advantage of eating lucerne, 'sensory properties' (35.4%) and 'vegetables spinach' (24.5%) were regarded as the most important disadvantage and association, respectively. The differences in lucerne knowledge of respondents from different demographic backgrounds indicated a need to inform and educate specific population groups in SA, on the benefits of consuming lucerne. The predictors, sensory qualities and synonyms, contributed significantly ($p<0.001$) to consumers' attitudes towards lucerne.

6.1 Introduction

Sensory properties of food are critically important determinants of food choice (Meiselman & MacFie, 1996). This is reflected by the multitude of sensory studies published, involving sensory preferences and acceptability of various food products. However, non-sensory factors have also been recognized for its contribution to food selection decisions of consumers (Santosa, Clow, Sturzenberger, & Guinard, 2013). According to Santosa et al. (2013), consumer behaviour, specifically the act of consumption, includes the acquisition, usage and disposal of products, ideas, services or events that provide values. The relationship between attitude and beliefs has been studied widely, although the relationship has remained somewhat unclear. However, attempts have been made to relate attitude to food consumption. These have led to the development of a framework of knowledge-attitude-practice (behaviour), which means that changes in behaviour can be brought about by increasing knowledge about a particular product.

Several studies reported that a higher awareness and knowledge about food had a positive influence on the attitude towards and consumption of food (Aertsens, Mondelaers, Verbeke, Buysse, & Van Huylenbroeck, 2011; Stobbelaar, Casimir, Borghuis, Marks, Meijer, & Zebeda, 2007). Consumers need to be confronted with more information about food products, in order to increase their knowledge thereof (Pieniak, Aertsens, & Verbeke, 2010). Peter and Olsen (2008) stated that consumers usually have general food product knowledge, information about the attributes or characteristics of food products and facts about the positive consequences or benefits of using food products. More knowledge about a certain food may increase the probability to buy the specific food, i.e., its appeal for non-users, as well as the level of consumption among existing consumers (Pieniak et al., 2010).

The more knowledge consumers have about products and services, the more likely they are to develop attitudes towards them, whether it is positive or negative. Consumers' attitude causes them to avoid using all available knowledge concerning a product or service, resulting in little knowledge use (Schiffman & Kanuk, 2010). As a result, consumers' attitude differ as their experience differ, causing them to behave differently in the same purchasing situation, in response to their positive or negative attitude. Consumers' attitude towards food and nutrition are important factors influencing food consumption behaviour in general, as well as sustainable food consumption (Pieniak et al., 2010). Verbeke (2008) stated that consumers must have a sufficient level of knowledge, based on reliable information, in order for information to have a favourable impact on their food choice. Nevertheless, there is a consensus that knowledge is a key construct in information processing and thus, in the consumer decision-making process.

Little is known about consumers' knowledge on and attitude towards lucerne. Hence, a combination of qualitative and quantitative methods was used in this study to understand consumer behaviour towards lucerne. The main goals of this study were firstly, to investigate consumers' knowledge from different demographic groups. Secondly, to investigate the role of health benefits, food safety risks, sensory qualities and synonyms on attitude towards lucerne, developing hypotheses via a conceptual model, and thirdly, to investigate consumers' attitude towards lucerne by means of thematic analysis (a qualitative measure).

6.2 Materials and methods

6.2.1 Data collection

Data were collected from consumers from Bloemfontein, FSP, during April 2013, implementing purposive sampling, because particular criteria were used (Maree & Pietersen, 2010). With purposive sampling, the researcher's own judgement may be used to select suitable respondents (Babbie, 2007), who may provide significant information regarding the research problem (Denscombe, 2003). A self-completion questionnaire was used, which was completed at the Food Science Division of the Department of Microbial, Biochemical and Food Biotechnology, UFS, Bloemfontein. This area was chosen, as it was on the campus and was both beneficial and practical to the researcher and respondents, regarding resources and controlling the environment. The demographic profile of the study population (n = 384), 187 male and 197 female consumers, is provided in Table 4.2 (Chapter 4).

6.2.2 Development of research hypotheses

In line with the Theory of Planned Behaviour (TPB) (Ajzen & Fishbein, 1980; Ajzen, 1991) and the method described by Michaelidou and Hassan (2008), hypotheses were developed via the conceptual model. The model included health benefits, food safety risks, sensory qualities and synonyms, as antecedents of consumers' attitudes towards lucerne (Figure 6.1). These antecedents were categorized as independent variables, while an attitude towards lucerne (a direct measurement) was categorized as the dependent variable. The role of each predictor, as forerunner of consumers' attitudes towards lucerne, was discussed in Chapter 2. Although the relationship between forerunners and attitude has not been uniformly supported in studies, the following hypotheses were investigated:

- H1: Health benefits will positively affect attitude towards lucerne;
- H2: Food safety risks will positively affect attitude towards lucerne;
- H3: Sensory qualities will positively affect attitude towards lucerne; and
- H4: Synonyms of lucerne will positively affect attitude towards lucerne.

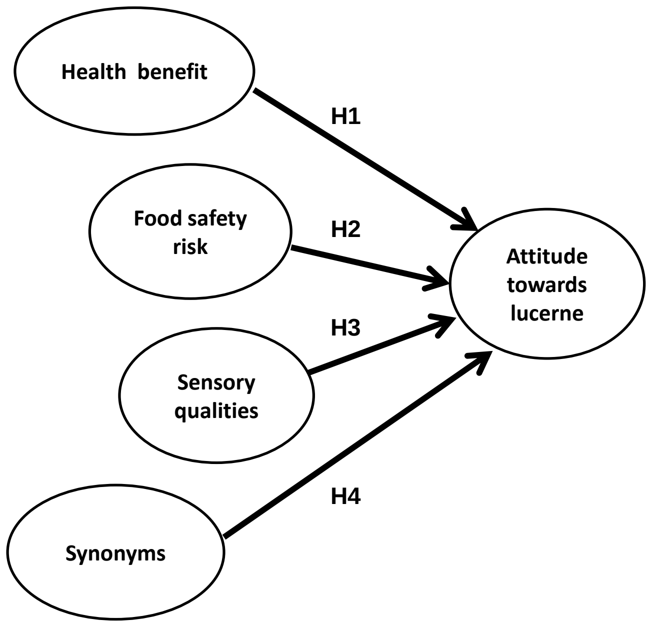


Fig. 6.1. Conceptual model (adapted from Michaelidou & Hassan, 2008).

6.2.3 Sampling method

The survey instrument was based on prior literature, with knowledge and attitude measures developed as recommended by Ajzen (1991), Francis et al. (2004), and Michaelidou and Hassan (2008). Part I ('Knowledge') (see annexure 12) included information regarding respondents' knowledge and consumption of lucerne, while part II ('Attitude') (see annexure 13) included Likert scale statements and three open ended questions: '*What do you believe are the advantages of eating lucerne?*'; '*What do you believe are the disadvantages of eating lucerne?*'; and '*Please indicate if there is anything else you associate with eating lucerne?*'. In part III ('Demographics') (see annexure 9), demographical information such as gender, age, population group, highest level of education and household income was collected. Demographical variables were used to detect whether these too could be linked to consumers' knowledge of lucerne.

6.2.4 Thematic analysis

Open-ended questions (qualitative data) were used in part II (see annexure 13) of the questionnaire, in order to obtain the most spontaneous answers from participants (Delport, 2005). Only 212 of the 384 participants were willing to answer the open-ended questions, as consumers were informed that their participation in this research was voluntary.

Furthermore, some of the consumers indicated that they were unsure or didn't know the answers.

Thematic analysis was used, in order to produce intelligible and interpretable data (Kruger, De Vos, Fouché, & Venter, 2005). This is a method to identify themes or categories in a document, the latter being a type of text (Trochim & Donnelly, 2008). Similar statements from participants (n = 212) were filed into categories and sub-categories, according to repeated occurrence (Strauss, 1987). All findings were tabulated and the categories were coded to quantitative data (Trochim & Donnelly, 2008).

6.2.5 Statistical analysis

All statistical procedures were conducted using the Statistical Package for Social Science version 17 (SPSS Inc., Chicago, IL, USA), by the Statistical Consultation Services of the North-West University, Potchefstroom. Descriptive statistics, such as frequency tables and means, were used to determine respondents' knowledge about and attitudes towards lucerne. The significance of differences of mean knowledge between demographic groups (dichotomous), i.e., gender, age, education, population group and income, were measured by the t-test for independent groups. This test determined whether there was a significant difference between two sets of scores. Significant levels should be smaller than 0.05, to indicate significant differences between variables (Jaafar, Lalp, & Mohamed, 2012).

The Cronbach alpha coefficient measured the internal consistency, to determine the reliability of the factor analysis, thus detecting the items' (health benefits, food safety risks, sensory qualities and synonyms) relatedness to each other. To guarantee construct validity, confirmatory factor analysis was used to show one-dimensionality of a variable (i.e., the numbers of factors, the variance explained of the extracted factor(s) and range of communalities). Factor analysis can be viewed as a data-reduction technique, since it reduces a large number of overlapping variables, to a much smaller set of factors (Chen & Chai, 2010).

The percentage of variance can be used to indicate convergent validity for each factor and should, ideally, explain at least half of the total variation among measured variables (Zikmund & Babin, 2007). Using the Pearson correlation coefficient, inter-correlations between variables were performed. Correlation determines the 'net strength' relationship between two continuous variables (Jaafar et al., 2012). In this study, correlation was used as the predicted change in the value of the dependent variable (i.e., attitude towards lucerne), for one-unit change in the independent variable (i.e., health benefits, food safety risks, sensory qualities and synonyms).

A multiple linear regression was used to explain the dependent variable, 'attitude direct measurement', by four other predictor variables (i.e., health benefits, food safety risks, sensory qualities and synonyms). Furthermore, this technique was applied to examine how two or more variables act together, to affect the dependent variable. It was also the equation that represented the best prediction of a dependent variable, from several independent variables (Jaafar et al., 2012). Results for reliability and factor analysis, descriptive statistics, the final model and hypothesis summary were tabulated according to the methods of Michaelidou and Hassan (2008).

6.3 Results and discussion

6.3.1 Consumers' knowledge of lucerne

According to the results in Table 6.1, 63.1% of the respondents knew what lucerne was. According to SSA (2012), 638 000 people are formally employed in the agricultural sector in SA. People, both directly and indirectly dependent on the agricultural sector for employment and income, amounts to approximately 8.5 million in SA and are familiar with forage crops such as lucerne. These findings could explain the more than average knowledge of lucerne.

A total of 77% of the respondents did not know what alfalfa was and 90.3% of the respondents had never consumed lucerne before the day of sensory testing (Table 6.1). Respondents' lack of knowledge of the name, alfalfa, could be explained by the fact that lucerne is known in Europe (except Spain and Portugal), SA, Australia and New Zealand as lucerne, but in the rest of the world as alfalfa (NLO, 2010). Furthermore, seed companies (such as K2 Agri and Pannar) and the National Lucerne Organisation (NLO) in SA is marketing this forage as lucerne.

Younger respondents (18 – 32 years) were significantly ($p<0.05$) more knowledgeable about lucerne than older respondents (33 – 65 years) (Table 6.2). These differences might be explained by the university environment, where most of the young respondents were recruited and who were busy with undergraduate or postgraduate studies at the UFS.

The white population group were significantly ($p<0.001$) more knowledgeable about lucerne than the Black African, Indian/Asian and coloured race groups. According to Vorster

Table 6.1.

Frequencies of responses to questions regarding knowledge of lucerne among respondents (*n* = 384).

Question	Frequency (n)	Percentage of sample (%)
Do you know what lucerne is?		
Yes	246	63.1
No	138	35.4
Do you know what alfalfa is?		
Yes	81	20.8
No	303	77.7
Have you ever consumed lucerne?		
Yes	32	8.2
No	352	90.3

Table 6.2.

Mean difference for knowledge of lucerne among different demographic groups of respondents (*n* = 384).

Variable	Mean	*t*-value
Gender		1.47
Male	6.8	
Female	6.3	
Age		2.59**
18 – 32 years	6.9	
33 – 65 years	6.2	
Population group		4.12***
White	8.0	
Other	6.3	
Education		4.1***
< Grade 12	5.5	
≥ Grade 12	6.8	
Income		-1.9
< R100 000	6.4	
≥ R100 000	7.1	

** = p<0.01; *** = p<0.001

(2007), knowledge differs from individual to individual. The food consumption in a community and household are influenced by their demographic and socio-cultural environments (e.g. culture, religion, wealth), leaving individuals with a specific knowledge regarding food consumption. Culture, specifically, influences eating habits and food preferences. Some households may go to immense measures to maintain their cultural identity (Viljoen, Botha, & Boonzaaier, 2005), leading to less informed consumers.

Respondents with a grade 12 or higher education were significantly ($p<0.001$) more knowledgeable, than those with qualifications below grade 12 (Table 6.2). Prattala, Berg and Puska (1992) observed that consumers, with a higher level of education, consumed more vegetables than consumers with a lower level of education. Lin (2002) proposed that more education leads to more information search, by increasing consumers' ability to identify, locate and assimilate relevant information. Moreover, consumers with higher education levels have more knowledge in general.

6.3.*2 The role of health benefits, food safety risks, sensory qualities and synonyms on consumers' attitudes towards lucerne*

Results from the reliability and factor analysis (Table 6.3) indicated that all six independent variables had eigenvalues greater than 1. Cronbach alpha coefficients were greater than 0.6 for all the factors, meaning that the scale scores for each of the dimensions were reliable. Due to the exploratory nature of this research, alpha values, greater than 0.6 for reliability estimates, were considered adequate (Chen & Chai, 2010).

Based on the descriptive data in Table 6.4, health benefits [mean (M) = 1.06, standard deviation (SD) = 0.65] became the most important criteria in consumers' attitude toward lucerne, followed by behavioural beliefs (M = 0.78, SD = 0.80) and food safety risks (M = 0.69, SD = 0.78). Attitude towards lucerne (M = 0.54, SD = 0.83) was average. In order to determine the most influencing factor predicting consumers' attitudes towards lucerne, relationships between all variables were determined through correlation analysis, before proceeding to regression analysis.

Table 6.4 depicts the r-value for the relationship between independent and dependent variables. The correlation readings indicated a 'no/negligible to very strong' relationship between variables (Quinnipiac University, 2013). Very strong positive correlations were noted between attitudes towards lucerne and sensory qualities ($r = 0.72$), and attitudes

Table 6.3.

Reliability and factor analysis results.

Variable	No. of items	Alpha (Corr)	% Variance extracted	Eigenvalue
Health benefits	6	0.79	51.79	3.11
Food safety risks	3	0.61	56.05	1.68
Sensory qualities	12	0.78	51.55	1.73
Attitude towards lucerne	4	0.89	74.84	2.99
Synonyms	2	0.73	52.50	1.77
Behavioural beliefs	6	0.87	60.40	3.62

Table 6.4.

Descriptive statistics of independent and dependent variables to determine factors, contributing towards consumers' attitudes in regard to lucerne.

Variable	Mean (SD)	1	2	3	4	5	6
1. Health benefits	1.06 (0.65)	1.00					
2. Food safety risks	0.69 (0.78)	0.38	1.00				
3. Sensory qualities	0.13 (0.60)	0.14	0.12	1.00			
4. Attitude towards lucerne	0.54 (0.83)	0.13	0.15	0.72	1.00		
5. Synonyms	0.03 (1.06)	0.17	0.09	0.62	0.69	1.00	
6. Behavioural beliefs	0.78 (0.80)	0.11	0.16	0.53	0.70	0.62	1.00

SD = Standard deviation

towards lucerne and behavioural beliefs (r = 0.70). Strong positive correlations existed between synonyms and attitudes towards lucerne (r = 0.69), synonyms and sensory qualities (r = 0.62), behavioural beliefs and synonyms (r = 0.62), and behavioural beliefs and sensory qualities (r = 0.53) (Table 6.4). Jaafar et al. (2012) stated that variables could be grouped into 'important determinants' and 'least important determinants' as influential factors, predicting consumers' attitudes towards lucerne. Therefore, these variables could be grouped as the 'important determinants' and seemed to have a significant influence on consumers' attitudes towards lucerne.

Moderate positive correlations were perceived between food safety risks and health benefits (r = 0.38), while no/negligible correlations were present between synonyms and health benefits (r = 0.17), behavioural beliefs and food safety risks (r = 0.16), attitudes towards lucerne and food safety risks (r = 0.15), sensory qualities and health benefits (r = 0.14), attitudes towards lucerne and health benefits (r = 0.13), sensory qualities and food safety risks (r = 0.12), behavioural beliefs and health benefits (r = 0.11), and synonyms and food safety risks (r = 0.09) (Table 6.4). These variables could be grouped as the 'least important determinants' and had no significant influence on consumers' attitudes towards lucerne.

Further examination, to determine the most significant factor influencing consumers' attitudes towards lucerne, was then conducted through multiple linear regression tests. As highlighted in Table 6.5, the B-values indicated the individual contribution of each predictor (health benefits, food safety risks, sensory qualities and synonyms) to the regression model. The relationship between each predictor and consumers' attitudes towards lucerne was explained, if the effects of all other predictors were held constant. If the coefficient was positive, there was a positive relationship between the predictor and consumers' attitudes towards lucerne, whereas a negative coefficient represented a negative relationship (Field, 2009).

The unstandardized coefficient (B; represents the gradient of the regression; Table 6.5) of food safety risks (0.07), sensory qualities (0.66) and synonyms (0.30) indicated that, as these predictors increased by one unit, consumers' attitudes towards lucerne will increase by 0.07, 0.66 and 0.30 units, respectively. The unstandardized error (SE B; Table 6.5) of each B-value indicated to what extent these values would vary across different samples. These standard errors were used to determine whether or not the B-value differed significantly from zero.

The standardized beta (ß; Table 6.5) coefficients were all measured in standard deviation units and were directly comparable; therefore, they provided a better insight into the 'importance' of a predictor in the model. This coefficient for sensory qualities and synonyms were close, 0.48 and 0.39, respectively. These values indicated that both variables had a comparable degree of importance in the model (this concurred with what the magnitude of the *t*-statistics implied) (Field, 2009). The standardized values of food safety risks (ß = 0.06), sensory qualities (ß = 0.48) and synonyms (ß = 0.39) indicated that, as these predictors increased by one standard deviation, consumers' attitudes towards lucerne increased by 0.06, 0.48 and 0.39 standard deviations, respectively.

The *t*-test indicated whether the B-values were significantly different from zero and measured whether the predictor made a significant contribution to the model. Therefore, if

Table 6.5.

Results of multiple linear regression to determine the most significant (p<0.001) factor influencing consumers' attitudes towards lucerne.

Variables	Dependent		Attitude		
Independent	B	SE B	β	t	Sign. Level
Health benefits	-0.03	0.04	-0.02	-0.57	p = 0.572
Food safety risks	0.07	0.04	0.06	1.79	p = 0.073
Sensory qualities	0.66	0.06	0.48	11.67	p < 0.001
Synonyms	0.30	0.03	0.39	9.60	p < 0.001
P value	0.000				
R^2	0.62				
F-ratio	156.32				

B = unstandardized coefficient; SE = Standard Error; β = Standardised coefficient; t = *t*-test; R^2 = Square root; F-ratio = ratio of the variance between groups to the variance within groups; NS = Not significant

the *t*-test, associated with the B-value, was significant (p<0.05), the predictor was making a significant contribution to the model. The smaller the value of significance (and the larger the value of *t*), the greater the contribution of that predictor (Field, 2009). From the *t*-statistics it was concluded that the sensory qualities (*t* = 11.67) and synonyms (*t* = 9.60) had a similar impact, whereas health benefits (*t* = -0.57) and food safety risks (*t* = 1.79) had less impact (Table 6.5).

The F-ratio tested the overall fit of the regression model to the set of observed data. It was a measure of how much the model improved the prediction of consumers' attitudes towards lucerne, compared to the level of inaccuracy of the model (Field, 2009). As the F-ratio was larger than one, the model was good and represented a significant effect.

The overall result for the regression model in Table 6.5 was significant (p<0.001). This indicated that all the independent factors were simultaneously significant to the dependent variables (Chen & Chai, 2010). It also was proof that consumers' attitudes towards health benefits, food safety risks, sensory qualities and synonyms contributed significantly (p<0.001) to the attitudes towards lucerne. The R^2 values (0.62) showed that the independent variables contributed 62% to the dimension of attitudes towards lucerne. From this analysis, both health benefits (p-value = 0.572) and food safety risks (p-value = 0.073) did not contribute significantly to the consumers' attitudes towards lucerne. However, sensory qualities and synonyms contributed significantly ($p<0.001$) to the dependent variable (attitude towards lucerne), with the significant value of 0.000.

In regard to hypotheses 1 and 2, the results indicated no significant relationship between consumers' attitudes towards health benefits and food safety risks, and their attitudes towards lucerne (Table 6.6). As for hypotheses 3 and 4, there was a significant ($p<0.001$) relationship between consumers' attitudes towards sensory qualities and synonyms, and their attitudes towards lucerne (Table 6.6). These differences might be explained by the fact that respondents believed that the sensory properties of lucerne were neither regarded as advantageous nor disadvantageous, and they could associate with lucerne, as noted in section 6.3.3. These beliefs could directly influence consumers' attitudes towards consuming lucerne. Aikman and Crites (2007) suggested that general sensory qualities (e.g. taste, smell) and specific sensory qualities (e.g. salty, greasy) will influence consumers' attitudes towards food products. These sensory qualities can be confirmed by the results obtained from external preference mapping in Chapter 5 of this thesis. The lucerne sample that was preferred most by the consumers, was 'SASS', because of its wet appearance, salty taste and spinach aroma. Both SBP and SBS were preferred by all consumers.

Table 6.6.

Summary of results to confirm hypotheses of the conceptual model.

Hypothesis	Predicted effect	Confirmed (Yes/No)
H1	Health benefits will positively affect attitude towards lucerne	No
H2	Food safety risks will positively affect attitude towards lucerne	No
H3	Sensory qualities will positively affect attitude towards lucerne	Yes
H4	Synonyms will positively affect attitude towards lucerne	Yes

Differences between lucerne attitudes and synonyms might be explained by the fact that the use of an unfamiliar (for SA consumers) synonym of lucerne on food labels, namely alfalfa, will influence consumers' attitudes towards consuming lucerne, as names, derived from different languages (e.g. Spanish-Arabic), may develop a more positive attitude towards lucerne products (Catchword Branding, 2013). For example, lucerne sprouts are sold in supermarkets as alfalfa sprouts, in combination with chick pea sprouts (product label provided in annexure 14) in SA (Pick ‘n Pay, 2014; Small, 2011).

6.3.3 Consumers’ attitudes towards lucerne (qualitative measure)

A summary of participants’ beliefs, regarding the advantages, disadvantages and associations of eating lucerne, are presented in Annexure 15. According to Ajzen (1991), an attitude consists of an organization of several beliefs, focused on a specific object or situation. Beliefs are defined as ‘salient information relevant to the behaviour’. An individual may hold several beliefs toward an object and with each of these beliefs, comes an evaluation (Dreezens et al., 2005). Furthermore, a belief can develop into an attitude, according to the strength of the feeling involved. The stronger the belief, the stronger becomes the attitude and the more likely it will affect behaviour (Wright, 2006).

The frequencies of responses to questions regarding advantages, disadvantages and associations of lucerne among participants are categorized in Table 6.7. Participants regarded ‘health general’ (40.1%), (e.g. *“I think it may play a vital part in healthy living and create a stable balanced diet for a healthy life*”; Annexure 15), ‘nutritious general’ (28.3%) (e.g. “*I do believe that it has a lot of nutritional value and can help consume vitamins and minerals necessary for healthy growth*”; Annexure 15) and ‘nutritious protein’ (12.7%) (e.g. “*It has high protein content as compared to other leafy vegetables*”; Annexure 15) as the most important advantages of eating lucerne (Table 6.7). In Chapter 3 of this thesis it was reported that cooked ‘SAS’ had a significantly ($p<0.001$) higher protein content than cooked SB. The protein content for ‘SAS’ raw and cooked was 4.61% and 4.04%, respectively, compared to SB raw (2.09%) and cooked (3.05%).

Michaelidiou and Hassan (2008) mentioned that health-conscious consumers are aware and concerned about their health, and are thus motivated to improve and/or maintain their health. Furthermore, they also strive to prevent ill health, by engaging in healthy behaviours and being self-conscious regarding health. In line with these developments, the healthy attributes of food and ways of eating are recurring themes in health policies, the media and everyday discussions around food (Niva & Mäkelä, 2007; Urala & Lähteenmäki, 2004). Munro and Small (1997) stated that, as the world’s most efficient protein-producing crop, humans may one day turn directly to lucerne for the bulk of their protein diet, rather than relying on first converting plant protein into meat from domestic animals.

Table 6.7.

Frequencies of responses to questions regarding advantages, disadvantages and associations of lucerne among participants (n = 212).

Category	Frequency (n)	Percentage of sample (%)
Advantages		
Health General	85	40.10
Health Body	11	5.20
Nutritious General	60	28.30
Nutritious Iron	12	5.70
Nutritious Protein	26	12.70
Nutritious Fibre	17	8.00
Natural	17	8.00
Vegetables General	17	8.00
Vegetables Spinach	13	6.10
Sensory Properties	6	2.80
Preparation	4	1.90
Costs	10	4.70
Availability	3	1.40
Disadvantages		
Health Risks General	18	8.50
Health Risks Allergies	7	3.30
Health Risks Digestion	6	2.80
Health Risks Chemical	5	2.40
Nutritious	3	1.40
Sensory Properties	75	35.40
Animal Feed	15	7.10
Positive Feedback	24	11.30
Preparations	7	3.30
Costs	5	2.40
Frequency of consumption	5	2.40
Associations		
Health	12	5.70
Vegetables Other/Plants	21	9.90
Vegetables Spinach	52	24.50
Sensory Properties	14	6.60
Serving Suggestions	8	3.80
Animal Feed	20	9.40

Other advantages included the belief that lucerne was not only regarded as a natural product (8%; Table 6.7) (e.g. "*I believe it is a natural plant that is nutritious*"; Annexure 15), but also as a healthier option (40.1%) than other foods (e.g. *"It is healthier than red meat"*; Annexure 15). Urala and Lähteenmäki (2004) confirmed that naturalness is quite visible in the hierarchical value maps of functional foods, indicating that consumers attach increasing importance to the way food is produced. Assessments of the naturalness of foods seem to be correlated with sensory appeal (Steptoe et al., 1995). Natural food is associated with better looks and better taste, compared to foods containing additives or artificial ingredients (MacFie, 2007).

Although lucerne is not yet classified, marketed or even known as a vegetable in SA, it would seem that 9.9% (Table 6.7) of the participants already regarded it as a vegetable (e.g. *"I believe it is like spinach and all the other vegetables which are good for my health"*; Annexure 15). A possible explanation for this optimism could be that people are paying more attention to the utilization of lucerne in the food industry, mainly due to its high nutritional contents (Hao et al., 2008; Lamsal et al., 2007). Flyman and Afolayan (2006) also stated that neglected crops and non-commercial foods are receiving renewed attention, with the recognition that they could become vehicles for improved nutrition and increased food supply. Despite people's preferences for familiar food products, they are also attracted to new food products, partly to overcome boredom and saturation effects. According to Schifferstein (2010), people prefer products with an optimal combination of typicality and novelty.

Examples of statements made regarding sensory properties (2.8%; Table 6.7) included e.g. "*Eating lucerne is a good idea because it tastes like spinach"* (see annexure 15). Results in Chapter 4 (section 4.3.1.2) indicated that 'SASP' (2.37) was significantly ($p<0.001$) higher in spinach aroma than 'WL525P' (1.81) and 'WL711P' (1.48). 'SA Standard stew' (3.74) was significantly ($p<0.001$) higher in spinach aroma than 'WL525P' (1.81) and 'WL711P' (1.48), and numerical higher in spinach aroma than 'WL525S' (3.56) and 'WL711S' (3.56). In Chapter 5 it was confirmed that 'SASS' was the lucerne sample most preferred by the consumers, because of its wet appearance, salty taste and spinach aroma. Urala and Lähteenmäki (2004) reported that attitude toward new foods may not only have an impact on willingness to try new foods, but may also influence the actual liking of a product.

Preparation was also considered by 1.9% (Table 6.7) of the participants, e.g. "*Cook it in many different ways"* (see annexure 15) as an advantage, although most of the consumers have never even prepared or cooked lucerne as a vegetable. While 2.4% of the participants made statements regarding lucerne being a more affordable food product, e.g. *"It might be a cheaper alternative to spinach and will add variety to the vegetable market"* (see annexure

15), other participants (1.4%) commented on the availability of lucerne, e.g. *“It will increase food availability in the market”* (see annexure 15). According to the PBPM (2011), lucerne is a major forage legume, grown on approximately 45 million hectares worldwide. Furthermore, lucerne is regarded as an affordable protein source (Davies, 2008).

Sensory properties (35.40%; Table 6.7) were regarded as a disadvantage of eating lucerne, e.g. “*Some people may not like the bitterness*” (see annexure 15). According to Cotto (2010), many consumers will initially not like lucerne’s taste and recommended that one should persevere, as it is definitely an acquired taste. D’Alvise et al. (2000) and Xie et al. (2008) stated that the use of lucerne protein concentrates in human food is limited by their negative sensory properties: dark colour due to polyphenols; granulous texture; poor solubility; and grassy taste. In Chapter 4, ‘SASP’ had the highest value for grassy aroma (section 4.3.1.1) of all the lucerne cultivars, but ‘WL711P’ showed the highest values for fibrous appearance (section 4.3.1.7) and mouthfeel (section 4.3.1.11), chewy mouthfeel (section 4.3.3.10), bitter taste (section 4.3.1.14) and aftertaste (section 4.3.1.16) (see annexure 11). Although consumers’ sensory acceptance for SBS was higher than SBP and all the lucerne cultivars (section 4.3.5), the numerical values for aroma (6.04), taste (6.02), texture (6.00) and overall acceptability (6.04) for ‘SASS’, were numerically higher than for ‘WL525S’ (5.94 / 5.90 / 5.49 / 5.75) and ‘WL711S’ (5.74 / 5.62 / 5.26 / 5.55) (section 4.3.5). Furthermore, results in Chapter 5 indicated that consumers in all three clusters in the PM preferred SB, but that ‘SASS’ was the preferred lucerne cultivar, having a wet appearance, salty taste and spinach aroma (Table 5.1).

Many misconceptions were stated as disadvantages by participants (Table 6.7), which could be due to consumers’ lack of knowledge. While 8.5% of the participants regarded the consumption of lucerne as a general health risk (e.g. “*You will get sick”*; Annexure 15), 3.3% of the participants believed that it could cause allergies (e.g. “*It might trigger some allergies for some people*”; Annexure 15).

Bora and Sharma (2011) elaborated on the phytochemical and pharmacological potential of lucerne, and reported that lucerne is used: to improve the memory; to cure kidney pain, cough, and sore muscles; as a rejuvenator; anti-diabetic; antioxidant; anti-inflammatory; antifungal; anti-asthmatic; antimicrobial; diuretic; galactagogue; and in central nervous system (CNS) disorders. The EFSA (2009), however, stated that the effects of the phytoestrogens, present in lucerne, on the fertility and the reproductive function of humans were difficult to establish and suggested that pregnant or lactating women be advised to avoid consumption of lucerne. According to Bora and Sharma (2011), a health care practitioner should be consulted prior to use of lucerne if undergoing hormone replacement therapy or taking birth control medication or using blood thinning medication.

Tharanathan and Mahadevamma (2003) stated that inclusion of lucerne in the daily diet has many beneficial physiological effects in controlling and preventing various metabolic diseases, such as diabetes mellitus, coronary heart disease and colon cancer. The earliest documented use of the lucerne genus was, in fact, as human food (Munro & Small, 1997), when, from 7 500 to 5 600 B.C., wild lucerne seeds and possibly leaves were consumed by humans in a village in South-Western Iran (Flannery, 1969). According to the EFSA (2009), lucerne is presently consumed in the human diet as food supplements and an ingredient in common foods (soups, salads). While Stramesi and Falabella (in Bolton, 1962) studied the nutritive value of lucerne leaves, Xie et al. (2008) confirmed that leaf protein has also been recognized by the Food and Agriculture Organization (FAO), as a potential source of high quality protein for human consumption, due to its abundance, nutritive value and absence of animal cholesterol. Following a request from the European Commission, the PDPNA was asked to deliver a scientific opinion on the safety of APC as food. As a food supplement, APC was recommended with a daily consumption of 10 g. They concluded that the coumestrol, isoflavone and L-canavanine content of APC, as well as the use thereof as a food supplement, at this proposed level, is of no safety concern (EFSA, 2009).

It would seem that 7.1% (Table 6.7) of the participants had difficulty consuming lucerne, because of it being an animal feed in SA, e.g. "*Eating the same products as animals"* (see annexure 15). MacFie (2007) stated that informing the public about new food products may often fail to increase acceptance, unless factors such as personal or societal benefits, and the values placed on these new food products, are also properly addressed. Personality variables may also shape consumers' attitudes toward new foods (Coulthard & Blissett, 2009). Some people have a stronger tendency to avoid new foods than other people, a phenomenon that has been labelled food neophobia. Both picky eating and neophobia are repeatedly associated with reduced consumption of fruit and vegetables. This finding has been in relation to both the amount of fruit and vegetables, and the variety of fruit and vegetables consumed.

Although most of the participants never prepared cooked lucerne leaves, 3.3% (Table 6.7) were concerned about the preparation of lucerne, e.g. "*They might be a bit unstable to heat"* (see annexure 15). Of the participants, 2.4% (Table 6.7) were concerned about the cost implication of consuming lucerne, e.g. "*Competing with livestock, will make lucerne expensive for farmers*" (see annexure 15). Eromosele et al. (2008) reported that the exorbitant cost of protein from animal sources has led to a growing interest in industrial applications of plant origin protein. Integrating underutilized crops into diets has been promoted as the most practical way to achieve sustainability, since such crops are efficient sources of several important micronutrients, both with respect to unit cost of production and per unit area of land (Flyman & Afolayan, 2006). Helms (2004) noted that a reduction of animal protein in meat-based diets would reduce the inefficient cereal demand. Consequently, the resources spared in the production of animal feeds could then be used for food production for the growing population and the conservation of natural resources.

Nevertheless, 11.3% (Table 6.7) of the participants provided positive feedback on the question requesting disadvantages of lucerne, e.g. "*None because it is a nutritious plant and it is healthy to eat green vegetables*" (see annexure 15). The fact that positive feedback is given for a negative (disadvantages) question, could imply that consumers have a generally positive attitude towards consuming lucerne. This positive attitude was further supported by the association of lucerne with the frequently consumed vegetable, spinach, by 24.5% of the participants (e.g. "*I associate it with eating green vegetables like spinach*" (see annexure 15). Babin and Haris (2009) stated that consumers in general have positive attitudes toward products that provide value. Lucerne was associated with vegetables by 9.9% of the participants, e.g. "*Wild leafy vegetables*" (see annexure 15). Serving suggestions (3.8%) made by participants, e.g. "*It may be eaten with porridge*" (see annexure 15), might also contribute to a positive attitude.

6.4 Conclusions

Consumers' knowledge and attitude towards lucerne were investigated by means of qualitative and quantitative methods, to understand consumer behaviour towards lucerne. Results from this chapter indicated that while 90.3% of the respondents had never consumed lucerne, 77% of the respondents did not know what alfalfa was. Older respondents (33 – 65 years) were significantly ($p<0.05$) less knowledgeable about lucerne than younger respondents (18 – 32 years). The white population group were significantly ($p<0.001$) more knowledgeable about lucerne than the Black African, Indian/Asian and coloured race groups. Respondents with a grade 12 or higher education were significantly ($p<0.001$) more knowledgeable, than those with qualifications below grade 12. These differences in lucerne knowledge of respondents from different demographic backgrounds indicated a need to inform SA consumers, on the benefits of consuming lucerne.

Descriptive statistics were used to determine the role of health benefits, food safety risks, sensory qualities and synonyms on consumers' attitudes towards lucerne. Very strong positive correlations were found between attitudes towards lucerne and sensory qualities, and attitudes towards lucerne and behavioural beliefs. The findings reported that sensory qualities and synonyms would positively affect consumers' attitudes towards lucerne and were important predictors in furthering researchers' understanding of their role towards human consumption of lucerne.

Consumers believed that the most important advantages when eating lucerne were health and nutrition, and they associated lucerne with vegetables and specifically spinach. This study also reported that participants did not perceive eating lucerne as having disadvantages, implying that consumers had a generally positive attitude towards consuming lucerne. These predictors and advantages should be used for product development, sustainable production and increased consumption of lucerne.

CHAPTER 7
GENERAL DISCUSSION AND CONCLUSIONS

To sustain SA's population growth in the future, it is necessary to produce more protein based foodstuffs. Presently, SA does not contribute to research on protein supplementation (Laula, 2010). When striving for sustainable food production and consumption systems, an analysis of dietary proteins from various sources (i.e. meat, dairy and plant) is an excellent starting point (De Boer, Helms, & Aiking, 2006). It is argued that a transition towards more plant protein based diets would simultaneously benefit consumers' health and nutritional needs.

The high costs of animal protein have led to a growing interest in the industrial applications of lucerne (generally known and used as animal feed) in SA. Goławska, Łukasik, Wójcicka and Sytykiewicz (2012) pointed out that the high nutritional value of lucerne is due to its substantial content of high quality protein and carbohydrates, suitable as nutritional food for human consumers.

A lack of available literature exists about lucerne as potential alternative food protein source for human consumption in SA. Neither the microbial content, sensory acceptance and attributes nor attitudinal behaviour of human consumers towards lucerne have been investigated. Furthermore, no research was undertaken to raise awareness of the potential utilisation of lucerne for human consumption, which could contribute to food security and human nutrition for sustainable development in SA.

Therefore, the aims of this thesis was to investigate the feasibility of introducing lucerne into the diet of consumers, as an alternative protein source, by means of chemical, microbial, and sensory characteristics, and secondly, to determine the attitudes of consumers towards lucerne.

The literature review in Chapter 2 focused on three components. The first component concentrated on the nutritional and microbial composition of lucerne, including the safety aspects and microbial diversity of lucerne. The second part established the need to investigate the sensory characteristics (such as aroma, appearance, mouthfeel, taste and aftertaste) of lucerne that play an important role in consumers' acceptance to consume lucerne, as well as the success of the final product. The last section focused on consumer behaviour, namely attitudes. Attitudes are important predictors of both consumer food choice and behaviour that needs to be investigated.

From the literature review, it was evident that SA consumers and researchers need to become aware of the nutritional and entrepreneurial potential of lucerne for consumption purposes in the future. Diversification of the lucerne industry will contribute to the unsatisfying diet status of SA consumers and target malnutrition. Furthermore, integrating lucerne into human diets can be promoted as the most practical and workable way to achieve food security in SA. Research efforts, on the evaluation of the potential of lucerne for industrial applications in the food field, will hold some challenges. However, these could contribute to food security, human nutrition, breeding programmes and convenient sources of income for effective and sustainable development in SA.

7.1 The chemical and microbial evaluation of lucerne

The objective of Chapter 3 was to investigate the chemical composition (degrees Brix, macro- and micro-minerals, protein, AA, dry matter, ash, fat, fibre, carbohydrates and energy content) and microbial content of three lucerne cultivars ('SA Standard', 'WL711' and 'WL525') and compare it to spinach beet (*Beta vulgaris* var. *cicla* L.) (a known vegetable). This was done to determine the nutritional potential of lucerne, as well as the cultivar for potential future research.

Lucerne cultivar 'SAS' was suggested for future investigation as it indicated a good Brix value (16.13%) that was significantly ($p<0.001$) higher than SB (4.77%). The highest values for Ca (2.59% / 2.15%), S (0.69% / 0.46%), Mg (0.51% / 0.33%), Cu (13.48 µg/g / 13.33 µg/g), DM (24.91% / 12.38%), ash (3.19% / 0.80%), fibre (6.47% / 1.63%), carbohydrate (16.75% / 6.80%) and energy (421.0 kJ/100 g / 247.33 kJ/100 g) were reported for raw and cooked 'SAS'. Furthermore, for average cooking losses, a positive gain were reported for K, Ca, S, Mg, Zn, Mn and Cu (7 out of the 10 minerals), after cooking 'SAS'. A high protein content for 'SAS' raw (4.61%) and 'SAS' cooked (4.04%) were also noted.

Of the three lucerne cultivars, 'SAS' raw indicated the highest level for AAs, His (0.25 g/100 g), Ile (0.24 g/100 g), Leu (0.39 g/100 g), Lys (0.39 g/100 g), Met (0.09 g/100 g), Phe (0.32 g/100 g), Val (0.29 g/100 g), Arg (0.35 g/100 g), Ser (0.27 g/100 g), Asp (0.77 g/100 g), Cys (0.11 g/100 g), Glu (0.56 g/100 g), Gly (0.25 g/100 g), Thr (0.27 g/100 g), Ala (0.35 g/100 g), Tyr (0.29 g/100 g), Pro (0.37 g/100 g) and HO-Pro (0.07 g/100 g).

Furthermore, 'SAS' raw and 'SAS' cooked indicated the same levels of epiphytic bacteria (TBC; log 6.85 cfu/g / log 1.10 cfu/g), coliform (log 4.88 cfu/g / log 1.00 cfu/g), LAB (log 3.03 cfu/g / log 1.00 cfu/g), yeast (log 3.06 cfu/g / log 1.00 cfu/g) and mould (log 3.95

cfu/g / log 1.10 cfu/g) counts. During the shelf-life study, marginal to satisfactory microbial levels were reported up to day seven for TBC (log 5.12 cfu/g), yeast (log 2.27 cfu/g) and mould (log 2.70 cfu/g) counts. Based on these findings, the nutritional and microbial shelf-life potential of lucerne compared well to the nutritional properties of SB.

7.2 Descriptive sensory analysis and consumer acceptability of lucerne

To suggest an animal feed, such as lucerne, for human consumption, although it may be safe and nutritious, could be pointless, if the sensory properties are less than optimal. Therefore, the objectives of Chapter 4 were firstly to determine sensory descriptive attributes of three lucerne cultivars by GDA, to confirm if 'SAS' should be further studied. Secondly, consumers' acceptance of lucerne was determined. This test included the same lucerne cultivars and one SB cultivar, to be evaluated for degree of liking for aroma, taste, mouthfeel and overall acceptability. It was hypothesized that lucerne could have a similar taste profile to SB.

Panellists generated and reached consensus on 13 (plain samples) and 15 (stew samples) sensory descriptors. Of all the samples described, 'SASS' showed the lowest value for potato aroma, and also the lowest value, of all the lucerne samples, for fibrous appearance, chewy and fibrous mouthfeel, bitter taste, and bitter and metallic aftertaste. Results from the GDA indicated that three stew samples (SB, 'SAS' and 'WL525') and SBP displayed the more 'positive' attributes, such as soft and wet appearance, spinach beet aroma and salty taste. 'WL711 stew' and the three plain samples ('SAS', 'WL525' and 'WL711') were associated with the more 'negative' descriptors, namely grassy aroma, fibrous and chewy texture, fibrous appearance, bitter taste, and bitter and metallic aftertaste.

It was concluded that 'SASS' was the most preferred lucerne cultivar. The numerical values for the degree of liking for aroma, mouthfeel, taste and overall acceptability for 'SASS' were higher than for 'WL525' and 'WL711'. The preference for 'SASS' was also higher than for 'WL525' and WL711'. Consumers also indicated that they were more likely to purchase 'SASS': within two weeks; one month; in supermarkets; use it as a substitute for SB; and get used to the taste of it. Not only was the average gain in taste, mouthfeel and overall acceptability for 'SAS' higher than the other two lucerne cultivars, but also the average gain to purchase the sample within two weeks, one month, in supermarkets and to get used to the taste of the sample.

The average gain for consumers' acceptance, preference and purchase intention between the lucerne plain and stew samples indicated that, while the level of acceptance for both SBP and SBS were the same, the plain lucerne samples improved in acceptance with the addition of potato and onion. Therefore, the method of preparation and addition of ingredients played an important role to make the lucerne samples more acceptable for consumers.

7.3 The relationship between consumer acceptability and descriptive sensory attributes of lucerne by using PM

Understanding the change and variations in flavour, that will occur during the development of lucerne products, is important in defining consumers' expectations of an acceptable product. The relationship between preference or acceptability and the descriptive sensory attributes of the lucerne cultivar will also be important. This will allow the product developer to concentrate on the attributes to develop the preferred product for consumers. According to Moskowitz et al. (2006), external PM uses sensory descriptive attribute ratings to locate the products on the maps.

In Chapter 5, the descriptive attributes for the three lucerne cultivars and one SB cultivar were related to consumer acceptability, by using external PM. As the relationship between consumer acceptability and descriptive sensory attributes of lucerne cultivars have never been studied in SA, it was necessary to perform PM on the plain and stew lucerne and plain and stew SB samples separately, and the plain and stew samples combined.

Different results were obtained when the samples were analysed both separately and combined. With the plain samples, all three consumer groups preferred the SBP sample. For cluster 1, 39.2% of the panellists preferred plain samples that were softer in mouthfeel and appearance. For cluster 2, 24.3% of the panellists preferred a wet appearance and salty taste, while for cluster 3, 36.4 % of the panellists preferred grassy and spinach aroma. These three clusters comprised of consumers of the female gender, 33 – 65 age group, Indian/Asian race group, primary completed education level group and > R750 000 income level group.

The results for the stew samples indicated that all three consumer clusters preferred the stew SB sample. For cluster 1, 19.9% of the panellists preferred the stew SB sample with potato and onion aroma, and soft appearance. For cluster 2, 11.4% of the panellists preferred a mushy and wet appearance and spinach aroma, while for cluster 3, 66.2% of the panellists preferred a salty taste. The closest lucerne cultivar to these attributes was 'SASS'. The three clusters comprised of females, 33 – 65 years old, Black Africans, with some primary education and earning R0 – R100 000 per year. All the consumers, except those with a primary completed education and who earned > R750 000 per year, indicated a higher acceptance for SBS than all the samples.

With the combined (plain + stew) samples, all three consumer clusters preferred SBP, SBS and 'SASS'. For cluster 1, 21.5% of the panellists preferred stew samples with a soft appearance and spinach aroma. 'WL525 stew' could be characterised by these attributes. For cluster 2, 45.4% of the panellists preferred the stew samples with a wet appearance, salty taste and spinach aroma. Spinach beet stew and 'SASS' could be characterised by these attributes. For cluster 3, 33.1% of the panellists preferred plain samples with a soft appearance and grassy aroma. Spinach beet plain could be characterised by these attributes. It was speculated that cluster 1 comprised of females, age 33 – 65 years, Indian/Asian, with some primary education and earning between R501 000 – R750 000 per year'. Cluster 2, comprising of males, 33 – 65 years old, Indian/Asian, with some high school education and earning R0 – R100 000 per year, showed the highest acceptance for 'SASS'. Also, all the consumers, except those with some primary education and who earned between R501 000 – R750 000 per year, indicated a high acceptance for 'SASS'. Furthermore, female consumers, age 33 – 65 years, Black Africans, with some primary education and earning R0 – R100 000 per year, indicated the highest acceptance for SBS. For cluster 3, consumers of the female gender, 33 – 65 age group, Indian/Asian race group, primary completed education level group and > R750 000 income level group, indicated a high acceptance for SBP. These results again confirmed that lucerne cultivar 'SAS' was the most acceptable lucerne cultivar.

7.4 Consumers' knowledge and attitudes towards lucerne

Little is known about consumers' knowledge on and attitudes towards lucerne. The main goals of Chapter 6 were firstly, to investigate consumers' knowledge from different demographic groups. Secondly, to investigate the role of health benefits, food safety risks, sensory qualities and synonyms on attitudes towards lucerne, developing hypotheses via a conceptual model, and thirdly, to investigate consumers' attitudes towards lucerne by means of thematic analysis (a qualitative measure).

From the results, it was evident that 77% of the respondents did not know what alfalfa was and 90.3% of the respondents had never consumed lucerne. Younger respondents (18

– 32 years) were significantly ($p<0.05$) more knowledgeable about lucerne than older respondents (33 – 65 years). The white population group were significantly ($p<0.001$) more knowledgeable about lucerne than the Black African, Indian/Asian and coloured race groups. Furthermore, respondents with a grade 12 or higher education were significantly ($p<0.001$) more knowledgeable, than those with qualifications below grade 12. These results indicated a need to inform and educate specific population groups in SA, on the benefits of consuming lucerne.

Descriptive statistics determined the role of health benefits, food safety risks, sensory qualities and synonyms on consumers' attitudes towards lucerne. With the correlation analysis, very strong positive correlations were noted between attitudes towards lucerne and sensory qualities ($r = 0.72$), while strong positive correlations existed between synonyms and attitudes towards lucerne ($r = 0.69$). Results from the regression model indicated that consumers' attitudes towards health benefits, food safety risks, sensory qualities and synonyms contributed significantly ($p<0.001$) to the attitudes towards lucerne. Sensory qualities and synonyms contributed significantly ($p<0.001$) to the dependent variable (attitude towards lucerne), with the significant value of 0.000. Therefore, sensory qualities and synonyms positively affected attitudes towards lucerne.

With the thematic analysis, participants regarded 'health general' (40.1%), 'nutritious general' (28.3%) and 'nutritious protein' (12.7%) as the most important advantages of eating lucerne. While 40.1% of the respondents regarded lucerne as a natural product (8%), 9.9% of the participants already regarded lucerne as a vegetable.

Sensory properties (35.4%) were regarded as a disadvantage of eating lucerne. While 8.5% of the participants regarded the consumption of lucerne as a general health risk, 7.1% of the participants had difficulty consuming lucerne, because of it being an animal feed in SA. Furthermore, 11.3% of the participants provided positive feedback on the question requesting disadvantages of lucerne. This optimistic response could imply consumers' positive attitude towards consuming lucerne. Respondents (24.5%) also associated lucerne with the frequently consumed vegetable, spinach. These predictors and advantages should be used for product development, sustainable production and increased consumption of lucerne.

7.5 Lucerne as a potential food product for human consumption

According to Sen et al. (1998), lucerne is of immense importance for both developed and lesser developed countries, as it contains a high amount of protein, and the yield of protein per unit area is greater than for any other known conventional crop (Sen et al., 1998).

This thesis proposes the use of lucerne as an alternative food product, because it can comply with South African consumer trends, stated by the Bureau for Food and Agricultural Policy (2012). Lucerne is described as: healthy; nutritious; sustainable, and convenient. It can promote wellbeing, is a natural product, shows indulgence and has an unconventional flavour as well as being suitable for home cooking. The PBPM (2011) stated that lucerne has certain key strengths: drought tolerance; versatility; perennial crop; wide adaptability; high yielding; and excellent feed quality.

In SA, as in other developing countries, an increase in the consumption of vegetable proteins could contribute to the prevention of both under-nutrition and diseases caused by over-nutrition. Studies done by De Witt (2005) and Van der Walt (2003) concluded that the most commonly perceived barriers to eating fruit and vegetables are affordability, availability, household taste preference, family influences and cultural beliefs. De Witt (2005) also found that the consumption of fruit or vegetables, in either urban or rural populations of SA, did not add up to 80 g/day. The consumption of leafy, brassica and fruity vegetables by urban children and adults are consistently less per day, compared to their corresponding rural populations. Another consistent phenomenon is that rural and urban children, aged 1 – 5 and 6 – 9 years, consume on average less fruit and vegetables per capita per day, than rural and urban adults. For many families, increasing the consumption of fresh fruits and vegetables means undoing existing food habits, which are not an easy process. With regard to taste, most resistance to vegetable and fruit consumption comes from children and, in some cases, men in the household (Love et al., 2001; Van der Walt, 2003).

Odhav, Beekman, Akula and Baijnath (2007) stated that underutilized vegetables are inexpensive and affordable to people in the poor segmentation and the advantage is that it is highly nutritious. It is important that the food industry make an effort to positively change the perception of consumers towards lucerne, because it is nutritious and can be more affordable than commercially produced vegetables. If these underutilized vegetables can be sold in supermarkets, the status of the leafy crops will increase and more consumers will purchase it, which will also lead to an increase in its demand (Voster, Jansen van Rensburg, Van Zijl, & Venter, 2007). According to Dweba and Mearns (2011), policy makers and research scientists have neglected the research on underutilized vegetables and therefore there is minimal information available. If researchers in the food industry make an effort with research on lucerne, the findings that are favourable can lead to new products. This will encourage the modern consumer, who is always looking for new foods to satisfy their needs. Investigating the uses and potential of lucerne is necessary to validate the benefits, e.g. medicinal properties, a good food source, and income. The use of lucerne by households of lower income, which are mostly large, can aid in achieving food security in SA (Dweba & Mearns, 2011).

This means that if the food industry starts to produce lucerne commercially, at a price lower than the current commercially produced vegetables, any type of family can afford it, without foraging for it in the wild. In contrast, Van der Walt, Loots, Ibrahim and Bezuidenhout (2009) stated that a suitable strategy would be to promote cropping of lucerne in home gardens of rural and urban settlements, to enrich diets that are high in starch. The institutional trade, such as restaurants, can add lucerne to their menus. In doing this, restaurants can give their clients the choice of an underutilized vegetable. Furthermore, food companies can also cultivated lucerne commercially and sell it either as a frozen or fresh vegetable, or as a ready-made meal. The full development of lucerne’s potential is hindered by a lack of awareness among consumers, as well as by a lack of capacity within a research community. A proposed cycle (see annexure 1) and lucerne recipes (see annexure 16) can be considered in promoting the increased use of lucerne.

7.6 Recommendations for future research

The following recommendations are stipulated for future studies:

1. phytochemical (total phenolic, flavonoid, proanthocyanidin, gallotannin, free gallic acid, iridoid) and antioxidant properties of SA lucerne cultivars;
2. chemical composition of SA lucerne cultivars, harvested at different morphological growth phases and in different soil types;
3. the effect of different processing methods on the chemical composition of SA lucerne cultivars;
4. expansion on the average mineral levels for more SA lucerne cultivars;
5. identification of the microbial population of SA lucerne cultivars relevant to food spoilage; and
6. promotion of lucerne for human consumption.

The promotion of lucerne leafy greens, as an underutilised food source for human consumption, must be considered, in order to increase the variety of plant sources available to consumers, and at the same time, to expand the utilization of the lucerne plant. As the consumption of lucerne could improve the protein intake of consumers, role-players in food-based programmes in SA should promote the cultivation and use of lucerne in rural and urban communities, in an effort to reduce malnutrition.

CHAPTER 8

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CHAPTER 9

SUMMARY

Lucerne (*Medicago sativa* L.) has not recently been investigated as an alternative protein source for human consumption in SA. The chemical (degrees Brix, macro- and micro-minerals, protein, AA, dry matter, moisture, ash, fat, fibre, carbohydrates and energy content) composition, microbial content, and shelf-life of three lucerne cultivars, 'SA Standard' ('SAS'), 'WL525' and 'WL711', were investigated. It was compared to SB (*Beta vulgaris* var. *cicla* L.), to identify the lucerne cultivar to use for future research purposes. The Brix content of the lucerne cultivars was significantly ($p<0.01$) higher than SB. Raw lucerne cultivars had significantly ($p<0.01$; $p<0.001$) higher Ca and S contents than raw SB. The K, S and Mn content for the raw lucerne cultivars were significantly ($p<0.001$) higher than the cooked cultivars. The protein content of the raw lucerne cultivars were significantly ($p<0.001$) higher than the raw SB. Cooked 'SAS' and 'WL525' had significantly ($p<0.001$) higher protein contents than cooked SB. Both raw and cooked 'SAS' had a significantly ($p<0.001$) higher carbohydrate and energy contents than both raw and cooked 'WL525', 'WL711' and SB. The lucerne cultivars contained higher values of essential and non-essential AA than SB. The total bacterial count, coliform count, lactic acid bacteria count and mould count conformed to standards, while cooking significantly ($p<0.001$) reduced the bacterial load. The shelf-life of cooked lucerne and SB samples were of satisfactory microbiological quality until day seven.

The sensory descriptive attributes of the three lucerne cultivars were determined, by using GDA, and consumers' acceptance (degree of liking for aroma, taste, mouthfeel and overall acceptability). 'SA Standard stew' showed the lowest value, of the lucerne samples, for fibrous appearance, chewy and fibrous mouthfeel, bitter taste, and bitter and metallic aftertaste. Principal component analysis showed that, three stew samples (SB, 'SAS' and 'WL525') and plain SB displayed 'positive' attributes, such as soft and wet appearance, spinach beet aroma and salty taste. 'WL711 stew' and three plain samples ('SAS', 'WL525' and 'WL711') were associated with 'negative' descriptors, namely grassy aroma, fibrous and chewy texture, fibrous appearance, bitter taste, and bitter and metallic aftertaste. 'SA Standard stew' was the most preferred lucerne cultivar, as it's values for degree of liking for aroma, mouthfeel, taste and overall acceptability were numerically higher than 'WL525' and 'WL711'.

External preference mapping indicated that three consumer clusters, with different lucerne preferences, were obtained by AHC. 'SA Standard stew' was the lucerne sample preferred by all the consumers, except those with some primary education and a yearly income of R501 000 – R750 000 per year, who indicated only a high acceptance for this sample.

While 63.1% of the respondents knew what lucerne was, 77% was unfamiliar with the term ‘alfalfa’ and 90.3% never consumed lucerne before. Younger respondents of the white population group, with a grade 12 or higher education, were significantly ($p<0.001$) more knowledgeable about lucerne. The predictors, sensory qualities and synonyms, contributed significantly ($p<0.001$) to consumers’ attitudes towards lucerne. While respondents believed that ‘health general’ (40.1%) was the most important advantage of eating lucerne, ‘sensory properties’ (35.4%) and ‘vegetables spinach’ (24.5%) were regarded as the most important disadvantage and association, respectively. The differences in lucerne knowledge of respondents from different demographic backgrounds indicated a need to inform SA consumers, on the benefits of consuming lucerne. Based on these findings, lucerne could be implemented as an alternative vegetable protein and therefore, cultivar ‘SAS’ was proposed for future studies and product development.

Keywords: lucerne, protein, SA Standard, spinach beet, sensory, knowledge, attitude

OPSOMMING

Lusern (*Medicago sativa* L.) word huidiglik nie as 'n alternatiewe proteïenbron vir menslike verbruik in SA ondersoek nie. Die chemiese (grade Brix-, makro- en mikro-mineraal-, proteïen-, aminosuur-, droë materiaal-, vog-, as-, vet-, vesel-, koolhidraat- en energie-inhoud) samestelling, mikrobiese inhoud en rakleeftyd van drie lusern kultivars, 'SA Standaard' ('SAS'), 'WL525', en 'WL711', is ondersoek. Dit is vergelyk met SB (*Beta vulgaris* var. *cicla* L.) om 'n geskikte kultivar vir toekomstige navorsingsdoeleindes te identifiseer. Die Brix-inhoud van die lusernkultivars was betekenisvol ($p<0.001$) hoër as SB. Rou lusernkultivars het 'n betekenisvolle ($p<0.01$; $p<0.001$) hoër Ca- en S- inhoud getoon as rou SB. Die K-, S- en Mn-inhoud vir die rou lusernkultivars was betekenisvol ($p<0.001$) hoër as die gekookte kultivars. Die proteïeninhoud van die rou lusernkultivars was betekenisvol ($p<0.001$) hoër as die rou SB. Gekookte 'SAS' en 'WL525' het 'n betekenisvolle ($p<0.001$) hoër proteïeninhoud gehad as gekookte SB. Beide rou en gekookte 'SAS' het 'n betekenisvolle ($p<0.001$) hoër koolhidraat- en energie-inhoud as beide rou en gekookte 'WL525', 'WL711' en SB gehad. Die lusern kultivars het hoër waardes vir essensiële en nie-essensiële aminosure as SB getoon. Die totale bakteriese-, kolivorme-, melksuurbakterieë- en skimmeltellings het aan standaarde voldoen, terwyl die kookproses die bakteriese lading betekenisvol ($p<0.001$) verminder het. Die rakleeftyd van gekookte lusern en SB monsters was van bevredigende mikrobiologiese kwaliteit tot en met dag sewe.

Die sensoriese beskrywende eienskappe van drie lusern kultivars is met GDA bepaal, asook verbruikers se aanvaarding ('hou van'-graad vir aroma, smaak, mondgevoel en algehele aanvaarbaarheid) van lusern. 'SA Standaard'-bredie het die laagste waarde, van al die lusernmonsters, vir veselagtige voorkoms, koubaar- en veselagtige mondgevoel, bitter smaak, en bitter- en metaalnasmaak getoon. *Principal component analysis* het getoon dat drie brediemonsters (SB, 'SAS' en 'WL525') en opgekookte SB 'positiewe' eienskappe getoon het, soos sag en nat voorkoms, SB -aroma en soutsmaak. 'WL711' bredie en drie opgekookte monsters ('SAS', 'WL525' en 'WL711') was met 'negatiewe' beskrywers, naamlik grasaroma, veselagtige en koubaar tekstuur, veselagtige voorkoms, bitter smaak, en bitter- en metaalnasmaak, geassosieer. 'SA Standaard' bredie was die mees gewenste lusernkultivar, aangesien die numeriese waardes vir die 'hou van'-graad vir aroma, mondgevoel, smaak en algehele aanvaarbaarheid hoër was as vir 'WL525' en 'WL711'.

External preference mapping het getoon dat drie verbruikersgroepe, met verskillende lusernvoorkeure, verkry is deur AHC. 'SA Standaard' bredie is deur al die verbruikers verkies, behalwe dié met gedeeltelike primêre opleiding en ‘n jaarlikse inkomste van R501 000 – R750 000 per jaar, wat net ‘n hoë aanvaarding vir hierdie monster getoon het.

Terwyl 63.1% van die verbruikers geweet het wat lusern was, was 77% onbekend met die term ‘alfalfa’ en 90.3% het nog nooit lusern geëet nie. Jonger respondente van die blanke populasiegroep, met ‘n graad 12 of hoër opleiding, het betekenisvol ($p<0.001$) meer kennis oor lusern getoon. Die antesedente, sensoriese kwaliteite en sinonieme het betekenisvol ($p<0.001$) bygedra tot verbruikers se houding teenoor lusern. Terwyl respondente geglo het dat ‘gesondheid algemeen’ (40.1%) die belangrikste voordeel was om lusern te eet, was ‘sensoriese eienskappe’ (35.4%) en ‘groente spinasie’ (24.5%) onderskeidelik as die belangrikste nadeel en assosiasie beskou. Die verskille in respondentkennis vanaf verskillende demografiese agtergronde, het ‘n behoefte getoon om SA verbruikers in te lig oor die voordele om lusern te eet. Gebasseer op hierdie bevindinge, kan lusern as ‘n alternatiewe groenteproteïen geïmplementeer word en daarom is kultivar ‘SAS’ voorgestel vir toekomstige studies en produkontwikkeling.

Sleutelwoord: lusern, proteïen, SA Standaard, spinasie beet, sensories, kennis, houding

ANNEXURE 1

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The utilisation of lucerne (*Medicago sativa*): a review

Annchen Mielmann
School for Physiology, Nutrition and Consumer Sciences, North-West University, Potchefstroom, South Africa

Abstract

Purpose – The aim of this review is to focus on the potential utilisation of lucerne which could contribute more effectively to human nutrition and food sustainability. It aims to explore a proposed cycle for the promotion of lucerne for human consumption. Furthermore it seeks to propose lucerne as a potential vegetable and to briefly discuss the chemical composition, protein application, safety and medicinal uses of lucerne.

Design/methodology/approach – A non-experimental approach, namely a systematic literature study, was used. During the study searches were done on the scientific databases Science Direct, Sabinet online, Emerald, EbscoHost and SpringerLink. Moreover internet searches were undertaken on the search engine, Google Scholar. Additionally, available textbooks and theses were consulted. Different combinations of keywords as well as synonyms for keywords were used during the searches. Some keywords used included lucerne, alfalfa and legumes.

Findings – The high costs of animal protein have led to a growing interest in the industrial applications of lucerne in developing countries as lucerne is a valuable source of protein, suitable to produce nutritional food for human consumers. The full development of lucerne's potential is hindered by a lack of awareness among consumers as well as by a lack of capacity within a research community. Therefore the key steps in the proposed cycle can be used to aid in the diversification of lucerne in the market place.

Originality/value – To the author's knowledge there exists a lack of previous reviews on lucerne. It is important for scientists to become aware of the nutritional and entrepreneurial potential and contribution for human consumption purposes in the future in order for the lucerne industry to diversify.

Keywords Lucerne, Alfalfa, Legume, Underutilised, Africa, Nutrition, Food crops

Paper type Literature review

Introduction

Recently, with the increase in the demand for green food, scientists are paying more attention to Lucerne or Alfalfa (*Medicago sativa* L.) in the food industry. Lucerne is a drought, hardy, perennial (Gault *et al.*, 1995), herbaceous forage legume (Lenné and Wood, 2004). It is characterised by a stout taproot, purple flower and spiral shaped pods (Dickinson *et al.*, 2010). Lucerne is grown in approximately 45 million hectares worldwide. It is the oldest plant grown solely for forage with livestock feeding dating back to more than 3,300 years ago (Pioneer Brand Products, 2011). Lucerne is a good source of slow release carbohydrates, proteins, minerals and vitamins (Tharanathan and Mahadevamma, 2003; Hao *et al.*, 2008; NLO, 2010). It contains between 15 and 22 per cent crude protein on a dry matter basis, as well as all the macro- and trace minerals and all the fat- and water-soluble vitamins (Scholtz, 2008), making it suitable for feeding to poultry and swine (Adapa *et al.*, 2007). Lucerne is mainly used to make hay and silage, but can also be used for grazing purposes, because of its high yield, quality and wide adaptability to different climates and soil types (NLO, 2010). Lucerne

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may be grown as a cover crop and often increases yield of succeeding crops such as potatoes, rice, cucumber, lettuce and tomatoes (Mustafa *et al.*, 2001).

In the last decade lucerne's popularity and potential for human consumption for specific health conditions has increased. The increase in media coverage of the health benefits of lucerne has generated a rise in consumer awareness and increased interest in research. It is therefore important for food scientists to become aware: of the nutritional and entrepreneurial potential and contribution for human consumption purposes in the future, in order for the lucerne industry to diversify; that lucerne can be promoted as the most practical and sustainable way to achieve sustainability in developed countries; that the exorbitant cost of protein from animal sources will continuously lead to a growing interest in industrial application of protein of plant origin to challenge the increasing demand for protein in the food and non-food market; and that fierce competition in the food market forces companies to differentiate and add value to their products through underutilised food ingredients such as lucerne. Research efforts on the evaluation of the potential of lucerne for industrial applications in the food field could contribute to food security, human nutrition, breeding programmes and convenient sources of income for effective and sustainable development. The main objective of this review is to propose a cycle to promote human consumption of lucerne as an alternative vegetable to enhance the contribution of lucerne to food security and human nutrition for sustainable development in developing countries.

Chemical composition of Lucerne

Table I illustrates the nutritional values of green forage, whole meal and leaf meal. When comparing the protein content, the leaf meal contains 20.4 g protein per 100 g (Aganga and Tshwenyane, 2003). Lucerne is a valuable source of vitamins A and E, it contains B carotene, thiamine, riboflavin, niacin, pantothenic acid, biotin, folic acid, choline, inositol, pyridoxine, vitamin B12 and vitamin K (Aganga and Tshwenyane, 2003). It is also an outstandingly good source of vitamin C (1.78 mg/g) (Levy and Fox, 1935)' but it loses 80 per cent on drying (Aganga and Tshwenyane, 2003), while vitamins B and D are present only in low concentrations. This is in agreement with Horst *et al.* (1984) who demonstrated the presence of vitamin D_2 and vitamin D_3.

Morrison (1961) reported that lucerne hay has a higher mineral content than grains like maise and wheat, because of the accumulation of minerals in the leaves during growth, soil washed onto the growing plants by rain, and dust settling on the roughage before it is stored. Levy and Fox (1935) examined the chemical composition and digestibility of 168 lucerne hay samples. With respect to macro minerals, it was reported that in lucerne hay, the sodium (Na) value varied the most, followed by chloride (Cl), manganese (Mg), potassium (K), calcium (Ca), phosphate (P) and sulphur

	Moisture (% /100 g)	Protein (g/100 g)	Fat (g/100 g)	Fibre (g/100 g)	Ash (g/100 g)
Green forage	80.0	5.2	0.9	3.5	2.4
Whole meal	7.5	16.0	2.5	27.3	9.1
Leaf meal	8.0	20.4	2.6	17.1	11.5

Table I. The nutritional value of fresh lucerne, whole meal and leaf meal

(S). Ca and Mg concentrations in lucerne hay are greater than for grasses at equivalent stages of maturity (Scholtz, 2008). In addition, about 8 mg of iron are present per g, or nearly double that found in spinach (Levy and Fox, 1935). The nutritional values of spinach and lucerne are illustrated in Table II (Dallas, 2011).

Maturity of the lucerne plant seems to have the biggest influence on the variation of chemical parameters as the quality of lucerne hay drops as the plant matures. This drop is largely due to the increasing stem: leaf ratio and to an increase in the fibre content of the stems. During the early flowering stage, the leaves contain a greater concentration of digestible nutrients, proteins, fats, fibre, total non-structural carbohydrates and other micronutrients than the stems. Stems have more sugars, fibre, K and Cl, therefore it is clear that the lucerne leaves contain more nutrients than the stems (Scholtz, 2008). Lucerne hay quality (nutritive value) varies considerably and is influenced by factors such as harvesting at specific physiological stages, climatic factors, edaphic factors such as soil conditions, leaf losses during haymaking, storage and feeding practice, disease and insects, weeds, lucerne cultivar, moisture content during storage, water supply and fertilisation (Scholtz, 2008).

Protein applications

Worldwide, legumes are a unique protein source in the diet. According to Tharanathan and Mahadevamma (2003) the dietary importance of legumes is expected to grow in the years, because of the protein demand of the increasing world population and the need of reducing the health risks and high costs related to consumption of animal food sources, especially in the pattern of low-income groups and developed countries. It is estimated that over 800 million people in the world are currently malnourished (Helms, 2004; Hishamunda and Ridler, 2006). These exorbitant costs of protein from animal sources have led to a growing interest in industrial application of legume proteins to challenge the increasing demand for protein in the food and non-food market. Lucerne protein is a good source for producing nutritious and functional food, which is extracted from lucerne leaf (NLO, 2010). Research has been conducted on preparation procedures, application, property and nutritional value of lucerne leaf protein. Proteins have been isolated from dry powders of six one-year old and two more than one-year-old Australian lucerne herbages with a sequential extraction procedure, where albumin was found to be the main protein and glutenin and globin were present in small amounts (Xie *et al.*, 2008).

Research efforts are now focusing on identification and evaluation of the potential of lucerne with good nutritional qualities that are well adapted to adverse environmental

Nutrient	Units	Spinach (per 100 g)	Alfalfa (per 100 g)
Energy	kcal	22	29
Energy	kj	92	121
Protein	g	2.86	3.99
Total lipid (fat)	g	0.35	0.69
Carbohydrate, by difference	g	3.5	3.78
Phosphorous, P	mg	49	70
Zinc, Zn	mg	0.53	0.92
Pantothenic acid	mg	0.0065	0.563

Table II. The nutritional value of lucerne compared to spinach conditions; high seed yield, pests and diseases resistant (Eromosele *et al.*, 2008). Lucerne biomass has potential biotechnological importance in the production of low fiber, juice-derived co-products such as particulate (chloroplastic) protein concentrates, soluble protein concentrates, carotenoids, vitamins, minerals, growth factors, pharmaceutical agents, cosmetic products, and transgenic enzymes (Sreenath *et al.*, 2001). A study by Xie *et al.* (2008) even illustrated antioxidant activity of peptides isolated from alfalfa leave protein (ALP) hydrolysate. The ALPs was believed to have high nutritive value in addition to antioxidant activity. Kapel *et al.* (2006) characterised an antihypertensive peptide from an Alfalfa white protein hydrolysate, which was produced by a continuous enzymatic membrane reactor.

Alfalfa protein concentrate (APC) has been recognised as a potential source of high quality protein (45-60 per cent) for human consumption (D'Alvise *et al.*, 2000). The use of plant protein concentrates derived from lucerne in human food is limited by their negative sensory properties: dark colour due to polyphenols, granulous texture, poor solubility and their grassy taste (D'Alvise *et al.*, 2000; Xie *et al.*, 2008) possibly because of the saponin content (Thacker and Haq, 2008). Food properties that may be influenced by these proteins include water holding capacity, emulsification, foaming, viscosity, gelation, and texture (Lamsal *et al.*, 2007).

APC (dose 10 g/day) has been tested for its nutritional value in several clinical trials carried out in countries such as Peru, India and Congo. The APC has been used since 1992 as a food supplement to combat malnutrition in several non-EU countries in the world with no reported deleterious effects. APC (320 tons) were consumed in 20 countries throughout the world and no deleterious effects of APC was reported by the various National Government Organisations that used the product. APC is also authorised in the USA, Canada and Mexico. Products are available in forms such as food supplements, drinks and chocolate bars, but mostly in the form of food supplements (capsules, tablets, powder). A survey was conducted in 25 Members States in 2006 with feedback from five countries confirming that lucerne is consumed in the human diet in the form of food supplements and as an ingredient in common foods (soups, salads). However, no quantitative data are available (EFSA, 2009).

Petin and Luzerne (2010a) stated that in response to lucerne's loss of competitiveness against rival products (such as oil seed cakes) and to falling subsidies, the lucerne industry had to diversify in Europe. Leaf protein has also been recognised by the Food and Agriculture Organization (FAO) as a potential source of high quality protein for human consumption due to their abundance of source, nutritive value, and absence of animal cholesterol (Xie *et al.*, 2008). With several years of research, supported by the European Union, the fractionation of lucerne juice to create nutritional and functional protein ingredients for the food and non-food industry (FRALUPRO) project was established and performed. Through advanced technology (i.e. fractionation of lucerne, extracting the protein) it could be demonstrated that lucerne contains a protein, called Rubisco (Petin and Luzerne, 2010a, b). Rubisco (ribulose 1, 5-bisphosphate carboxylase), also called Fraction-I protein, accounts for up to 30-70/100 g of soluble lucerne leaf proteins (SLP) (Lamsal *et al.*, 2007). Rubisco accounts for approximately 2 per cent of the total dry matter fraction of lucerne and also helps plants to convert energy from the sun. It could profitably replace soya as a source of protein in food. At the moment, almost 80 per cent of the plant proteins in food come from soya, but none of them covers humans' nitrogen and amino acid requirements. By contrast, Rubisco contains all the essential amino acids which humans need and is closer to milk proteins. It also has foaming and emulsifying properties, which could be applied in foods (Petin and Luzerne, 2010a).

Lucerne for human consumption

Lucerne has been incorporated in recipes such as lucerne: puree sauté, tortilla, tea, croquettes, soufflé, pudding, soup, as well as raw and cooked salad (Bolton, 1962; NLO, 2010). Green lucerne has been used as a human food in parts of Russia, China and America, whilst in South Africa it is occasionally used as a substitute for spinach. Lucerne meal was incorporated into a special "cereal mixture" and used in the feeding of small children (Levy and Fox, 1935). Chinese farmers also treated it as a kind of vegetable (Hao *et al.*, 2008). In a study by Hao *et al.* (2008) lucerne was utilised to increase the protein, dietary fiber, mineral and vitamin content of wheat flour. Lucerne is one of the most popular sprouts available on the European markets. They are consumed often raw or slightly cooked in salads and sandwiches or as decorative appetisers (Peñas *et al.*, 2009).

According to Cotto (2010) many consumers will not like the taste of lucerne and (might also create the sense that it is burning the tongue tip) and they recommend that one should persevere as it is definitely an acquired taste. Studies done by Kalač *et al.* (1996) and Sen *et al.* (1998) indicated that saponins are responsible for the bitter taste in lucerne. Recent sensory test trials performed with human volunteers using saponins isolated from lucerne aeriel parts, showed that zahnic acid tredismoside is responsible for the bitter taste (Oleszek, 2002).

Medicinal uses

Lucerne is usually milled and taken in the form of pills to control and prevent certain medical conditions (NLO, 2010). In Chinese and Hindu societies, physicians used young lucerne leaves to treat disorders related to the digestive tract, arthritis and water retention. They made a cooling poultice from the seeds for boils. In Colombia it is used to treat coughs. It may have a therapeutic effect on gastric ulcers and has been used in the treatment of kidney stones. Lucerne can help to reduce exhaustion and nervous agitation. Due to lucernes' phytoestrogen (isoflavones and coumarins) content, it is reputed to regulate menstrual cycles and to stimulate milk flow in breastfeeding women. Experiments carried out by clinical nutritionists in 1982 showed that eating lucerne helped to protect monkeys from atherosclerosis that were on a high-cholesterol diet. They also proved the effectiveness of lucerne in decreasing blood-cholesterol levels. Lucerne seeds can be added to the diet to help normalise serum cholesterol concentrations in patients with type II hyperlipoproteinemia (HLP) (Mölgaard *et al.*, 1987).

Lucerne may also be used as a traditional plant treatment for diabetes. The administration of lucerne in the diet (62,5 g/kg) and drinking water (25 g/l) reduced the hyperglycaemia of streptozotocin-diabetic mice. The results demonstrated the presence of antihyperglycaemic, insulin-releasing and insulin-like activity in lucerne. Leaves of lucerne are used traditionally as a tea to treat diabetes in South Africa. The use in human subjects of lucerne as an antidiabetic agent has, at least in part, been attributed to its relatively high Manganese content (Gray and Flatt, 1997).

Lucerne as a potential vegetable

According to the 2010 MyPyramid vegetable subgroups from the ten fruit and vegetables subgroups proposed by Pennington and Fisher (2010), legumes contain ten vegetables: black eye peas, green peas, lentils, lima beans, kidney beans, mung beans, navy bean, pigeon peas, pinto beans and soybeans. However, these are classified as pulses or edible legumes, while lucerne is a forage legume (Lenné and Wood, 2004). According to Sen *et al.* (1998) lucerne is of immense importance for both developed and lesser-developed countries as it contains a high amount of protein, and the yield of protein per unit area is greater than for any other known conventional crop (Sen *et al.*, 1998). This review also proposes the use of lucerne as an alternative vegetable as lucerne can comply with the South African consumer trends stated by the BFAP (2010). Lucerne: is healthy, nutritious, and convenient and can promote wellbeing; is a natural product; shows indulgence and has an unconventional flavour; is a sustainable crop; shows simplicity; possesses natural goodness, and can be used for home cooking. The Pioneer Brand Products lucerne manual (Pioneer Brand Products, 2011) states that lucerne has certain key strengths: drought tolerance; versatile; perennial crop; wide adaptability; high yielding; and excellent feed quality.

In South Africa, as in other developing countries, an increase in the consumption of fruit and vegetables could contribute in the prevention of both under nutrition and diseases caused by over nutrition. Studies done by De Witt (2005) and Van der Walt (2003) concluded that the most commonly perceived barriers to eating fruit and vegetables are affordability, availability, household taste preference, family influences and cultural beliefs. De Witt (2005) found that the consumption of none of the varieties of fruit or vegetables in either the urban or rural populations of South Africa adds up to 80 g/day. The consumption of leafy, brassica and fruity vegetables are consistently less by urban children and adults per day, compared to their corresponding rural populations. Another consistent phenomenon is that rural and urban children aged 1-5 and 6-9 years consume on average less fruit and vegetables per capita per day than rural and urban adults. For many families, increasing the consumption of fresh fruits and vegetables means undoing existing food habits, which are not an easy process. With regard to taste, most resistance to vegetable and fruit consumption came from the children and, in some cases, the men in the household (Love *et al.*, 2001; Van der Walt, 2003).

Odhav *et al.* (2007) states that underutilised vegetables are inexpensive and affordable to people in the poor segmentation and the advantage is that it highly nutritious. It is important that the food industry make an effort to positively change the perception of consumers towards lucerne because it is nutritious and can be more affordable than commercially produced vegetables. According to Voster *et al.* (2007) if these underutilised vegetables can be sold in supermarkets the status of the leafy crops will increase and more consumers will purchase it, that will also lead to an increase in the demand for it. Dweba and Mearns (2011) means that policy makers and research scientists have neglected the research on underutilised vegetables and therefore there is minimal information available. If researchers in the food industry make an effort with the research on Lucerne, the findings that are favourable can lead to new products and the consumer are always looking for new foods to satisfy their needs. Investigating the uses and potential of lucerne is necessary to validate the benefits like the medicinal properties, good source of food, as well as income. The use of the lucerne by households of lower income and that are mostly large can help with the achieving of food security for them (Dweba and Mearns, 2011).

This means that if the food industry starts to produce lucerne commercially at a price lower than the current commercially produced vegetables any type of family can afford it and it will not be necessary for them to seek for it in the wild. In contrast states Van der Walt (2009) that a suitable strategy would be to promote cropping of lucerne in home gardens of rural and urban settlements to enrich their diets that is high in starch. The food industry in terms of restaurants can add lucerne to their menus. The restaurant can give their client the choice to choose from an underutilised vegetable. Companies in the food industry can also cultivate lucerne commercially and sell it either as a frozen vegetable, fresh vegetable or a ready-made meal that just have to be heated in the microwave. Awareness campaigns are necessary to help create awareness amongst all consumers to promote lucerne.

Safety of lucerne

For nearly all plant foods it is very difficult to obtain scientific data that document their history of safe consumption, even though they may have been eaten for several hundreds of years (Knudsen *et al.*, 2008). There are certain precautions to be considered when consuming lucerne. Consumption should be avoided during pregnancy and breastfeeding. A physician should be consulted if using hormone replacement therapy (HRT). Avoid in case of diabetes of systemic lupus erythematosus (SLE), and with the use of anticoagulants such as Wafarin or Herparin (NLO, 2010).

Lucerne sprouts in large quantities can cause a form of anaemia. In the US lucerne herb and seed are generally recognised as safe (GRAS) (EFSA, 2009), however isolated cases of Salmonella and *Escherichia coli* contamination in lucerne sprouts have been reported.

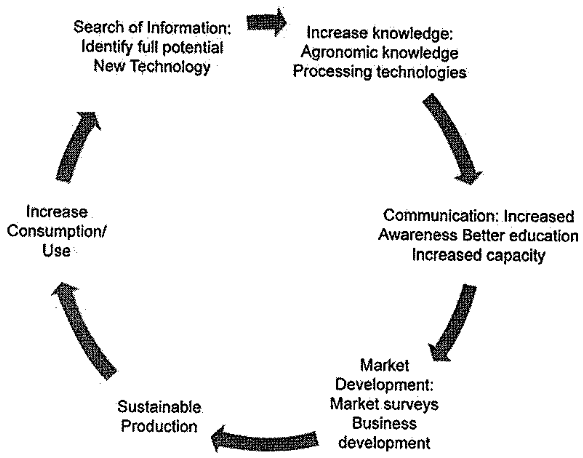
Following a request from the European Commission, the Panel on Dietetic products, Nutrition and Allergies was asked to deliver a scientific opinion on the safety of APC as food. In this report the applicant proposed the use of APC as a food supplement with a recommended consumption of 10 g per day. The Panel concluded that the coumestrol, isoflavone and L-canavanine content of APC as well as the use of APC as a food supplement at the proposed level of 10 g per day is of no safety concern (EFSA, 2009).

Proposed cycle for the promotion of lucerne

The proposed cycle (Figure 1), adapted from Jaenicke and Höschle-Zeledon (2006) outlines the outstanding challenges and opportunities to be considered in promoting increased use of lucerne; growing urbanisation, international trade, climate change, and health care, are major areas of direct relevance. Although lucerne have great potential for helping to address important concerns in these areas, the full development of their potential is hampered by lack of awareness in society, as well as by lack of relevant capacity within the research community. The key steps in the cycle are:

(1) Search for information on lucerne by identifying the full potential thereof and new technological methods applied on Lucerne.

(2) Knowledge will be increased by scientific research, mapping of agronomic knowledge, investigating processing technologies applicable to lucerne and documentation of existing information that at present may not be accessible.



Source: Medicago sativa

Figure 1.
Proposed cycle for the promotion of lucerne (*Medicago sativa)*

(3) Communication of the gathered information will raise the awareness of the general public about the value of lucerne; relevant information will lead to more educated consumers and will also contribute to capacity building amongst poor communities itself.

(4) Improved market development through market surveys, practical interventions, entrepreneurial training and fostering of public-private partnerships at all stages of the value chain to improve the supply and demand of lucerne and their products.

(5) The prerequisites for continuing targeted research and development activities that will use and further increase the knowledge base include increased capacity amongst the poor communities and a better-educated generation. This will produce an iterative process that will enable sustainable growth in the production of and demand for lucerne and lucerne products.

(6) Finally, assuming that appropriate policies, increased demand, sustainable production and increased/better supply coincide, the goal of increased consumption/use of lucerne to address the overall development problem will be reached. To achieve this, however, the aforementioned potential barriers need to be overcome; to this end, more effort, interaction and lobbying will be required (Jaenicke and Höschle-Zeledon, 2006).

Conclusion

Lucerne is generally known as a roughage source for livestock, but is becoming readily available in many parts of the world for human consumption, as it is a valuable source of protein, which could contribute to sustainable food development in developing countries. The key steps in the proposed cycle can be used to make scientists aware of the protein applications and nutritional benefits as well as to aid in the diversification of lucerne in the market place for human consumers.

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Corresponding author
Annchen Mielmann can be contacted at: Annchen.Mielmann@nwu.ac.za

ANNEXURE 2

Information on the sampling phases of lucerne.

1. Mid-vegetative:

a. Stem length 16–30 cm.
b. No visible buds, flowers, or seed pods.
c. As the stems continue to develop, axillary branch formation begins with the appearance of one or two leaves in the axil.
d. Development of axillary leaves is more pronounced in the mid-portion of the stem than at the base or apex.

2. Late vegetative:

a. Stem length greater than/equal to 31cm.
b. No visible buds, flowers, or seed pods.
c. Elongating branches are often found in the axils of the leaves at this stage.
d. It may be possible to feel buds at the growing apex, but they are not visible without peeling back the enclosing leaves.

ANNEXURE 3

Summary of the chemical and microbial results of the raw and cooked lucerne cultivars used in this study.

	'SA Standard'		'WL525'		'WL711'	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
Brix			+			
Chemical						
Macro-minerals						
P					+	+
K				+	+	
Ca	+	+				
S	+	+				
Mg	+	+				
Micro-minerals						
Zn				+	+	
Fe			+			+
Mn					+	+
Na			+	+		
Cu	+					+
Average cooking loss		+		+		+
Dry Matter	+	+				
Moisture			+	+		
Ash	+					+
Protein					+	+
Fat		+			+	
Fibre	+					+
Carbohydrate	+	+				
Energy	+	+				
Amino acids	+					
Microbial						
TBC				-	-	
Coliforms		-		-	-	-
LAB		-		-	-	-
Yeasts		-		-	-	-
Moulds				-	-	
Shelf life (d)						
TBC				2		4
Yeasts		2		3		6
Moulds		1		2		6

+ = highest value; - = lowest value; d = n days with the lowest counts

ANNEXURE 4

KEURINGSVRAELYS / *RECRUITMENT QUESTIONNAIRE*

Van: / *Surname*:		
Naam: / *Name*:		
Geboortedatum: / *Date of birth*:		
Geslag: / *Gender*:	**Manlik / *Male***	**Vroulik / *Female***
Nasionaliteit: / *Nationality*:		
Posadres / Fisiese adres: / *Postal address / Physical address:*		
Tel no:		
Sel no: / *Cell no*:		
Epos adres: / *Email address*:		
Naam van werkgewer: / *Name of employer:*		
Beroep: / *Occupation*:		
Hoogste kwalifikasie verwerf: / *Highest degree obtained*:		

Voltooi SLEGS as u huidiglik 'n student is: / *Complete ONLY if you are currently a student:*

Studente no: / *Student no:*	
Departement: / *Department*:	
Fakulteit: / *Faculty*:	
Graad geregistreer: / *Degree registered*:	
Jaar geregistreer vir huidige kwalifikasie: / *Year registered for current qualification*:	

Beantwoord die volgende vrae: / *Answer the following questions*:

Rook u? / *Do you smoke?*	**JA / *YES***	**NEE / *NO***
Vroue: Is u swanger? / *Females: Are you pregnant?*	**JA / *YES***	**NEE / *NO***
Vroue: Borsvoed u?/ *Females: Are you breastfeeding?*	**JA / *YES***	**NEE / *NO***
Lei u aan enige voedselallergene en/of ander voedselintoleransies? / *Do you suffer from any food allergies and/or food intolerances?*	**JA / *YES***	**NEE / *NO***

Indien JA, spesifiseer asseblief: / ***If YES, please specify:***		
Gebruik u enige medikasie wat u sintuie, veral smaak en reuk mag affekteer? / ***Do you use any medication which may affect your senses, especially taste and smell?***	**JA /** ***YES***	**NEE /** ***NO***
Indien JA, spesifiseer asseblief: / ***If YES, please specify:***		
Lei u aan enige kroniese of tydelike kondisies wat u sensoriese persepsie mag affekteer (insluitende diabetes, bloedsuikertekort, hipertensie, mondsiektes, sinusitus)? / ***Do you suffer from any chronic or temporary conditions that may affect your sensory perception (Including diabetes, hypoglycemia, hypertension, oral disease, sinusitis)?***	**JA /** ***YES***	**NEE /** ***NO***
Indien JA, spesifiseer asseblief: / ***If YES, please specify:***		
Gebruik u anti-koagulante of enige medikasie wat die koagulasie/klonting van bloed verhoed bv. ***Warfarin*** **of** ***Heparin®? / Do you use any anticoagulants or any medication that prohibits the coagulation/clotting of blood e.g. Warfarin*** **or** ***Heparin®?***	**JA /** ***YES***	**NEE /** ***NO***
Indien JA, spesifiseer asseblief: / ***If YES, please specify*****:**		
Het u enige valstande/kunsgebit of enige ander tandprostese (bv. draaitjies, plaaitjies) wat u sensoriese (bv. tekstuur en mondgevoel) persepsie kan affekteer? / ***Do you have any dentures or any dental prostheses (e.g. braces, dental plates) that may affect your sensory (e.g. texture and mouthfeel) perception?***	**JA /** ***YES***	**NEE /** ***NO***
Indien JA, spesifiseer asseblief: / ***If YES, please specify*****:**		
Is u tans op 'n streng gereguleerde dieet? / ***Are you currently on a restricted diet?***	**JA /** ***YES***	**NEE /** ***NO***
Indien JA, verduidelik asseblief: / ***If YES, please explain*****:**		
Eet u blaargroentes? / ***Do you eat leafy vegetables?***	**JA /** ***YES***	**NEE /** ***NO***

Indien JA, spesifiseer asseblief: / *If YES, please specify*:

Hoe gereeld eet u blaargroentes in ‘n maand? / *How often do you eat leafy vegetables in a month?*

Hoe gereeld eet u uit in ‘n maand? / *How often do you eat out in a month?*

Hoe gereeld eet u wegneemetes in ‘n maand? / *How often do you eat take-aways in a month?*

Wat is u gunsteling voedsel? / *What is your favourite food?*

Wat se voedsel sal u verkieslik nie eet nie? / *Which food will you preferably not eat?*

Wat se voedsel eet u glad nie? / *What food do you not eat at all?*

Is jou vermoë om tussen smaak en reuk te kan onderskei: / *Is your ability to distinguish between taste and smell:*	**Bo-gemiddeld / *Better than average***	**Gemiddeld / *Average***	**Ondergemiddeld / *Worse than average***

Beskryf asseblief van die merkbare smake in mayonnaise. / *Please describe some of the noticeable tastes in mayonnaise.*

Beskryf asseblief van die merkbare smake in spinasie. / *Please describe some of the noticeable tastes in spinach.*

Beskryf asseblief van die merkbare smake in filter/geperkoleerde koffie of gewone tee. / *Please describe some of the noticeable tastes in filter/percolated coffee or regular tea.*

Omkring asseblief u keuse: Koffie / Tee / *Please circle you choice: Coffee / Tea*

Dankie vir u tyd. / *Thank you for your time.*

ANNEXURE 5

Telkaart / *Score Sheet*

Naam: / *Name*:__________________ Datum: / *Date*:_____________

Tyd: / *Time*: ______ Stel no: / *Set no*:_____

- U ontvang gekookte lusernmonsters. / *You receive cooked lucerne samples.*
- Evalueer die monsters vir die volgende kenmerke: / *Evaluate the samples for the following attributes:*
  0 = Geen / *None*; 10 = Ekstreem / *Extreme*
- Begin deur: 1) die monster te ruik; 2) die monster te proe; en 3) na die monster te kyk. / *Start by: 1) smelling the sample; 2) tasting the sample; and 3) looking at the sample.*
- Onthou om 'n slukkie water te neem voor en tussen die proe van die monsters./ *Remember to take a sip of water before and in between tasting the samples.*

0 1 2 3 4 5 6 7 8 9 10

None **Extreme**

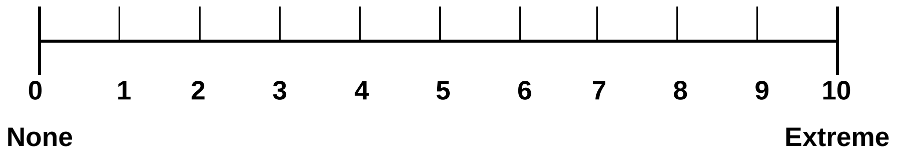
SAMPLE: 5 g fresh lucerne leaves cooked in filtered water for 10 min. and drained. Serving temperature = 40 °C.	
AROMA	
Grassy – Aroma associated with freshly cut grass. Freshly cut grass (4)	
Spinach – Aroma associated with cooked spinach beet. Cooked spinach beet (2)	
APPEARANCE	
Soft – Freshly sliced mushrooms that appear not to have resistance in mouth. Freshly sliced mushrooms (4)	
Wet – The appearance of cooked spinach beet after drained. Cooked spinach beet (4)	
Fibrous – The appearance of raw spinach beet. Raw spinach beet (7)	
MOUTHFEEL	
Soft – Feeling of food that does not provide resistance in mouth. *Smash* (3)	
Chewy – The mouthfeel of raw SB that does not disintegrate when chewed. Raw spinach beet stem (6)	
Fibrous – Amount of stringy fibers present in a spinach beet stem. Cooked spinach beet stem (7)	
TASTE	
Salty – Basic salt taste associated with NaCl. 0.15% NaCl solution (1)	
Bitter – Basic bitter taste associated with caffeine. 0.02% caffeine solution (1)	
AFTERTASTE	
Salty – Basic salt aftertaste associated with NaCl. 0.15% NaCl solution (1)	
Bitter – Basic bitter aftertaste associated with caffeine 0.02% caffeine solution (1)	
Metallic – Basic metallic aftertaste associated with ferrous sulphate. 0.15 % ferrous sulphate heptahydrate solution (1)	

Telkaart / *Score Sheet*

Naam: /*Name*:__________________ Datum: / *Date*:_____________

Tyd: / *Time*: ______ Stel no: / *Set no*:_____

- U ontvang gekookte lusernmonsters. / *You receive cooked lucerne samples.*
- Evalueer die monsters vir die volgende kenmerke: / *Evaluate the samples for the following attributes:*
  0 = Geen / *None*; 10 = Ekstreem / *Extreme*
- Begin deur: 1) die monster te ruik; 2) die monster te proe; en 3) na die monster te kyk. / *Start by: 1) smelling the sample; 2) tasting the sample; and 3) looking at the sample.*
- Onthou om 'n slukkie water te neem voor en tussen die proe van die monsters. / *Remember to take a sip of water before and in between tasting the samples.*



SAMPLE: 5 g fresh lucerne leaves cooked in filtered water for 10 min., drained and mixed with potato and onion. Serving temperature = 40 °C.	
AROMA	
Onion – Aroma associated with freshly cut onions. Freshly cut onions (3)	
Potato – Aroma associated with cooked warm potato. Cooked warm potato (1)	
Spinach – Aroma associated with cooked spinach beet. Cooked spinach beet (2)	
Grassy – Aroma associated with freshly cut grass. Freshly cut grass (4)	
APPEARANCE	
Soft – Freshly sliced mushrooms that appear not to have resistance in mouth. Freshly sliced mushrooms (4)	
Wet – The appearance of cooked spinach beet after drained. Cooked spinach beet (4)	
Mushy – The appearance of mashed potatoes. Potatoes boiled (60 min) overcooked and mashed (5)	
Fibrous – The appearance of raw spinach beet. Raw spinach beet (7)	
MOUTHFEEL	
Mushy – The mouthfeel of mashed potatoes. Potatoes boiled (60 min) overcooked and mashed (5)	
Chewy – The mouthfeel of raw SB that does not disintegrate when chewed. Raw spinach beet stem (6)	
Fibrous – Amount of stringy fibers present in a spinach beet stem. Cooked spinach beet stem (7)	
TASTE	
Salty – Basic salt taste associated with NaCl. 0.15% NaCl solution (1)	
Bitter – Basic bitter taste associated with caffeine. 0.02% caffeine solution (1)	
AFTERTASTE	
Bitter – Basic bitter aftertaste associated with caffeine. 0.02% caffeine solution (1)	
Metallic – Basic metallic aftertaste associated with ferrous sulphate. 0.15 % ferrous sulphate heptahydrate solution (1)	

ANNEXURE 6

RECRUITING QUESTIONS

1. Are you wholly or partially responsible for household grocery purchases?

YES	1	Continue
NO	2	Close

2. Do you eat vegetables?

YES	1	Continue
NO	2	Close

3. Which of the following leafy vegetables have you bought in the last 3 months?

1	Spinach	
2	Lettuce	
3	Broccoli	
4	Cauliflower	
5	Swiss Chard	
6	Brussels Sprouts	
7	Turnip	
8	None of the above	

If none of the above, close

4a. Which types of leafy vegetables do you eat/consume

4b. Which types of leafy vegetables do you use regularly in cooking, salads etc…

		4a. Eat regularly	4b. Use regularly
1	Spinach		
2	Lettuce		
3	Broccoli		
4	Cauliflower		
5	Swiss Chard		
6	Brussels Sprouts		
7	Turnip		

If one of the vegetables used and eaten, continue

5a. How often do you eat leafy vegetables?

5b. How often do you use leafy vegetables?

		5a. Eat	5b. Use
1	Everyday		
2	Not every day, but 2-6 times a week		
3	Once a week		
4	Once every two weeks		
5	Once every three weeks		
6	Once a month		
7	Less often		

Respondents must be regular users of leafy vegetables i.e. they must have used and eaten leafy vegetables within the last 2-3 weeks.

ANNEXURE 7

INLIGTINGSDOKUMENT/ *INFORMATION DOCUMENT*

Navorsingstitel: / ***Research title:***

Die chemiese, mikrobiese en sensoriese evaluering van lusern (*Medicago sativa* **L.) vir menslike verbruik en aanvaarbaarheid. /** ***The chemical, microbial and sensory evaluation of lucerne (****Medicago sativa* **L.*) for human consumption and acceptability.***

Goeiedag/ *Good day*,

Ek, **ANNCHEN MIELMANN,** PhD student in die Departement Mikrobiese, Biochemiese en Voedselbiotegnologie, Afdeling Voedselwetenskap, aan die Universiteit van die Vrystaat, is besig om navorsing te doen oor lusern/alfalfa *(Medicago sativa* L.*)* vir menslike verbruik. Navorsing is slegs die proses waardeur die antwoord op 'n vraagstuk verkry word. In hierdie studie wil ek die aanvaarheid en houding van verbruikers teenoor lusern toets, deur verbruikers aan verskillende luserndisse te laat proe. / *I,* ***ANNCHEN MIELMANN****, PhD student in the Department of Microbial, Biochemical and Food Biotechnology, Food Science Division at the University of the Free State, am doing research on lucerne/alfalfa (Medicago sativa L.) for human consumption. Research is just the process to learn the answer to a question. In this study I want to test the acceptance and attitudes of consumers towards lucerne by tasting different lucerne dishes.*

Ek nooi u uit om aan hierdie navorsingstudie deel te neem. U deelname sal 'n waardevolle bydrae maak in die groeiende aanvraag na nuwe voedselproteïenbronne in die wêreld, asook verdere navorsing wat sal voortspruit om lusern as 'n toekomstige proteïen gebasseerde groentebron te gebruik. / ***I am inviting you to participate in this research study.*** *Your participation will make a valuable contribution in the growing demand for new food protein sources in the world, as well as further research that will emerge to use lucerne as a future protein based source of vegetable.*

DOEL / *AIM*

Om met behulp van chemiese, mikrobiese en sensoriese metodes die uitvoerbaarheid van die bekendstelling van lusern in die dieet van verbruikers as alternatiewe proteïen groentebron te ondersoek en die houding van verbruikers teenoor lusern gebasseerde voedselprodukte te bepaal. / *To investigate, by means of chemical, microbial and sensory methods, the feasibility of the introduction of lucerne into the diet of consumers as an alternative protein vegetable source and to determine the attitude of consumers towards lucerne based food products.*

AGTERGROND / *BACKGROUND*

Dit word ‘n werklikheid dat Suid-Afrikaanse verbruikers proteïenryke voedsel bennodig wat ‘n beduidende rol in sekere toestande van gesondheid, insluitende die behoud van gesondheid en voorkoming van siektes, kan speel. Lusern is belangrik vir ontwikkelende lande soos Suid-Afrika, aangesien dit ‘n hoë hoeveelheid proteïene bevat. Teen die jaar 2020 sal die wêreld se voorsiening van proteïene moet verdubbel. Dit is opvallend dat Suid-Afrika nie ‘n rol speel nie in, of ‘n bydrae maak tot, die voorsiening van proteïene in die wêreld nie. Dit is daarom belangrik om Suid-Afrikaanse navorsers bewus te maak van die geleentheid wat hulle het om ‘n waardevolle bydrae in hierdie groeiende aanvraag na proteïene in die wêreld te maak, soos die inkorporering van meer plant proteïen peulgroente, soos lusern, in die dieet van Suid-Afrikaanse verbruikers. / *It is becoming a reality that South African consumers need protein rich food that can play a major role in certain states of health, including maintaining health and preventing disease. Lucerne is important for developing countries, such as South Africa, as it contains a high amount of protein. By the year 2020 the worlds’ protein supplementation will have to double. What is striking is that South Africa does not play a role, or make a contribution, towards protein supplementation in the world. It is, therefore, important to make South African researchers aware of the opportunity that they have, to make valuable contributions in this growing protein demand of the world, such as incorporating more plant protein legumes, like lucerne, in South African consumers’ diets.*

Aangesien daar ‘n toenemende belangstelling in die gebruik van lusernproteïene in voedselprodukte is, is dit belangrik dat die smaak en geur van hierdie proteïenvoedsel vir verbruikers smaaklik is. Daarom is dit nodig om die aanvaarbaarheid van lusern, asook verbruikers se houding teenoor lusern, te evalueer, aangesien houding ‘n psigologies neiging is wat uitgedruk word deur ‘n spesifieke entiteit te evauleer, met ‘n mate van voorkeur of afkeur teenoor voedselprodukte. / *As there is an increasing interest in the use of lucerne proteins in food products, it is important that the taste and flavour of these protein foods are appealing to consumers. It is therefore necessary to evaluate the acceptance of lucerne, as well as consumers’ attitudes towards lucerne food products, as attitude is also a psychological tendency that is expressed by evaluating a particular entity with some degree of favour or disfavour towards food products.*

VERWAGTE DUUR VAN STUDIE / *EXPECTED DURATION OF STUDY*

Die toets sal plaasvind by die Voedselwetenskap Afdeling se sensoriese laboratorium (Kamer 7.110B) in die Landbougebou by die Universiteit van die Vrystaat, Bloemfontein kampus, vanaf 9 April 2013 tot 26 April 2013. / *The test will take place at the Food Science*

Division's' sensory laboratory (Room 7.110B) in the Agricultural building at the University of the Free State, Bloemfontein campus on from 9 April 2013 to 26 April 2013.

PROSEDURE / *PROCEDURE*

Daar sal van u as deelnemer verwag word om lusern- en spinasiemonsters te proe en vraelyste te voltooi, om verbruikers se aanvaarbaarheid, voorkeur, kennis en houding teenoor lusern te toets, tydens een sessie van een uur lank. Tydens die projek sal relevante inligting aan die deelnemer verskaf word, rakende die projek./ *It will be expected of you, as participant, to taste lucerne and spinach samples and to fill out questionnaires to test consumers' acceptance, preference, knowledge and attitudes towards lucerne during one session of one hour long. The participant will be given pertinent information on the study while involved in the project.*

RISIKOS EN VOORDELE / *RISKS AND BENEFITS*

Risikos - U mag nie aan die navorsing deelneem nie, as u:

- enige voedselallergene het, bv. allergies vir neute;
- swanger is;
- borsvoed;
- antikoagulante soos *Warfarin* or *Heparin*® gebruik nie.

Risks - You may not participate in the research, in case you:

- *have any food allergies, e.g. allergic to nuts;*
- *are pregnant;*
- *are breast feeding;*
- *using anti-coagulants such as Warfarin or Heparin®*

Enige deelnemer wat nie voldoen aan die bogenoemde vereistes nie, sal van die studie geëlimineer word. / *Any respondent who does not comply with the above mentioned requirements, will be eliminated from the study.*

Voordele – Lusern is 'n natuurlik gesonde en voedingsryke produk. Dit is 'n waardevolle bron van proteïene, essensiële aminosure, vitamiene A, D, E? en K, mineraalelemente (Fe, Ca en P) en dieetvesels. Gekookte luserne kan gebruik word vir tuiskook en kan maklik voorberei word. Lusern kan ook maklik gekweek word en is 'n droogtebestande, volhoubare gewas. / *Benefits - Lucerne is a natural, healthy and nutritious product. It is a valuable source of proteins, essential amino acids, vitamins A, D, E and K, mineral elements (Fe, Ca and P)* *and dietary fibers. Cooked lucerne can be used for home cooking and can be prepared easily. Lucerne can also be easily grown and is a drought resistant, sustainable crop.*

Die volgende faktore moet ook oorweeg word wat die deelnemer kan bevoordeel:

- U moet in voedsel geïnterreseerd wees;
- U moet genoeg tyd beskikbaar hê om die toetste af te lê;
- Vir u deelname in die studies al u vergoeding ontvang.

The following factors must also be taken into consideration that might benefit you as a participant:

- *You must be interested in food;*
- *You must have enough time available to take the tests;*
- *You will receive a reimbursement for your participation in this research.*

VERTROULIKHEID / *CONFIDENTIALITY*

Daar sal gepoog word om persoonlike inligting vertroulik te hou. Absolute vertroulikheid kan nie gewaarborg word nie, aangesien persoonlike inligting bekendgestel mag word soos vereis deur die wet. Organisasies wat u navorsingsrekords mag inspekteer en/of kopieer vir kwaliteitsversekering, en data analises, sluit groepe in soos die Ektiekkomitee van die Fakulteit van Gesondheid, Universiteit van die Vrystaat, of navorsers van hierdie studie wat onderworpe is aan professionele geheimhouding./ *Efforts will be made to keep personal information confidential. Absolute confidentiality cannot be guaranteed as personal information may be disclosed if required by law. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Ethics Committee of the Faculty of Health, University of the Free State or investigators of this study who are subject to professional secrecy.*

DEELNAME / *PARTICIPATION*

Deelname is vrywillig en die weiering om deel te neem behels geen straf of verlies van voordele, waaraan die deelnemer andersins geregtig is nie.U mag enige tyd deelname onderbreek sonder straf of verlies van voordele, waaraan die deelnemer anders geregtig is. As u besluit om die studie te onderbreek voordat die toetse klaar is, staan dit u vry om so te doen, sonder enige vooraf waarskuwing en u sal geen ongemak of verlies van voordele, wat u gedurende die insluitingstyd geniet het, ervaar nie. Hoewel, as u besluit om die studie te onderbreek, moet u die navorsers dadelik in kennis stel. Die navorsers het ook die reg om die studie te onderbreek as hulle enige newe-effekte opspoor wat skade kan versoorsaak. As resultate gepubliseer word, kan dit lei tot groepsidentifikasie. / *Participation is voluntary,* *and refusal to participate will involve no penalty or loss of benefits, to which the subject is otherwise entitled. You may discontinue participation at any time without penalty or loss of benefits, to which the subject is otherwise entitled. If you decide to interrupt the study before the tests are finished, you can do so without any prior warning and you will neither experience any inconvenience nor lose any benefit you have enjoyed at the inclusion time. However, if you decide to interrupt the study, you should inform the researchers immediately. The researchers also reserve the right to interrupt your study if any adverse reactions are detected that can cause harm. If results are published, this may lead to cohort identification.*

MEDIESE HULP / ***MEDICAL ASSITANCE***

As ‘n deelnemer enige newe-effekte toon, na die inname van gekookte lusern, moet die hoofnavorser, Annchen Mielmann, dadelik gekontak word om die nodige reëlings te tref vir die deelnemer om die relevante mediese behandeling van ‘n gekwalifiseerde mediese praktisyn te ontvang. Alkoholverbruik, kaffeïeninname en rook is verbode op die dag van sensoriese toetsing en die gebruik van sulke middels moet aangemeld word./ *If a respondent shows any side effects after consuming the cooked lucerne, such a person should contact the head researcher, Annchen Mielmann, immediately in order to make the necessary arrangements for the respondent to receive the relevant medical treatment from a qualified medical practitioner. Alcohol consumption, caffeine intake and smoking are prohibited on the day of sensory testing and should be reported if any such substances were used.*

Indien u enige vrae of opmerkings het voor, gedurende of na die studie, kan u die navorser enige tyd skakel. Die navorser sal na u omsien gedurende die studie./ *If you have any questions or remarks before, during or after the study, you can contact the researcher at any time. The researcher will attend to you throughout the study.*

Hierdie navorsingstudie is goedgekeur deur die Etiekkomitee van die Fakulteit Gesondheidswetenskappe, Universiteit van die Vrystaat (Telefoonnommer: 051– 405 2812). / *This research study has been approved by the Ethics Committee of the faculty of Health Sciences, University of the Free State (Telephone number: 051 – 405 2812).*

Groete / *Regards*,

ANNCHEN MIELMANN

Navorser / *Researcher*

Selfoon: / *Cell phone*: 076 393 0670, E-pos: / *Email*: Annchen.Mielmann@nwu.ac.za

ANNEXURE 8

TOESTEMMING TOT DEELNAME AAN NAVORSING /
CONSENT TO PARTICIPATE IN RESEARCH

PROJEKTITEL: / *PROJECT TITLE:*

Die chemiese, mikrobiologiese en sensoriese evaluering van lusern (_Medicago sativa_ **L.) vir menslike verbruik en aanvaarbaarheid. / *The chemical, microbial and sensory evaluation of lucerne (*** _Medicago sativa_ ***L.) for human consumption and acceptability.***

- U is versoek om aan 'n navorsingstudie deel te neem./ *You have been asked to participate in a research study.*
- U is oor die studie ingelig deur:/ *You have been informed about the study by:* ANNCHEN MIELMANN.
- U kan ANNCHEN MIELMANN enige tyd kontak by 076 393 0670, indien u vrae oor die navorsing het of as gevolg van die navorsing beseer is. / *You may contact ANNCHEN MIELMANN at 076 393 0670, any time if you have questions about the research or if you are injured as a result of the research.*
- U kan die Sekretariaat van die Etiekkomitee van die Fakulteit Gesondheidswetenskappe, Universiteit van die Vrystaat by telefoonnommer (051) 405 2812 kontak, indien u enige vrae het oor u regte as 'n deelnemer. / *You may contact the Secretariat of the Ethics Committee of the Faculty of Health Sciences, University of the Free State at telephone number (051) 405 2812, if you have questions about your rights as a research subject.*
- U deelname aan hierdie navorsing is vrywillig en u sal nie gepenaliseer word of voordele verbeur as u weier om deel te neem of besluit om deelname te staak nie. / *Your participation in this research is voluntary, and you will not be penalized or lose benefits if you refuse to participate or decide to terminate participation.*
- As u instem om deel te neem, sal 'n ondertekende kopie van hierdie document, sowel as die inligtingsblad, wat 'n geskrewe opsomming van die navorsing is, aan u gegee word. / *If you agree to participate, you will be given a signed copy of this document, as well as the participant information sheet, which is a written summary of the research.*
- Die navorsingstudie, insluitend die bogenoemde inligting, is verbaal aan my beskryf. Ek begryp wat my betrokkenheid by die studie beteken en ek stem vrywillig in om deel te neem. / *The research study, including the above mentioned, has been verbally described to me. I understand what my involvement in the study means and I voluntarily agree to participate.*
- U is bewus daarvan dat u onder geen omstandighede mag deelneem aan die navorsingstudie, indien u: 1) enige voedselallergene het; 2) swanger is; 3) borsvoed; of 4) anti-koagulante soos *Warfarin* or *Heparin*® gebruik nie. / *You are aware of the fact that you may under no circumstances participate in the research study if you: 1) have any food allergies; 2) are pregnant; 3) are breastfeeding; or 4) are using anticoagulants such as Warfarin or Heparin®.*

________________________ ______________________

Handtekening van deelnemer /
Signature of participant

Datum/ *Date*

ANNEXURE 9

DEMOGRAPHICS

Please complete the following by indicating with a X.

1.

What is your gender?	
Male	1
Female	2

2.

How old are you?	
Younger than 18 years	1
18 to 32 years	2
33 to 65 years	3
Older than 65 years	4

3.

What is your population group?	
Black African	1
Coloured	2
Indian or Asian	3
White	4
Other	5

4.

What is your highest level of education?	
No formal education	1
Some Primary	2
Primary completed	3
Some High	4
Matric (Grade 12)	5
Tech diploma/degree	6
University degree	7
Other[1]	8
Unspecified	9

5.

What is your yearly income of the household?	
R0 – R100 000	1
R101 000 – R500 000	2
R501000 – R750 000	3
More than R750 000	4

[1] E.g. College or Services Sector Education and Training Authority (SSETA) diploma

ANNEXURE 10

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA
UFS·UV
NATURAL AND AGRICULTURAL SCIENCES
NATUUR- EN LANDBOUWETENSKAPPE

Departement Mikrobiese, Biochemiese en Voedsel Biotegnologie

Posbus 339
BLOEMFONTEIN 9300
Suid-Afrika

Tel: **051-401 9096**
Faks: **051-401 9335**
E-Pos: **bothmac@ufs.ac.za**

CONSUMER TEST: Lucerne

Gender ____________ **Date** ____________

Age ____________ **Tray** ____________

INSTRUCTIONS
Please indicate with a X how much you like or dislike the product OVERALL. Open container and smell sample before tasting.

Sample code:

Aroma

1	2	3	4	5	6	7	8	9
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Taste

1	2	3	4	5	6	7	8	9
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Mouthfeel

1	2	3	4	5	6	7	8	9
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Overall acceptability

1	2	3	4	5	6	7	8	9
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

PREFERENCE

INSTRUCTIONS
Please indicate with a X how often you will be willing to eat the tested product.

Sample code:

1	2	3	4	5
I will never eat it	I will only eat it when no other food is available	I will eat it occasionally (once per month)	I will eat it often (once per week)	I will eat it every day

PURCHASE INTENTION

INSTRUCTIONS
Please indicate with a X how much it is unlikely or likely that you agree with the following statements. No answers are considered incorrect, but honesty will be appreciated.

Sample code:

1. How likely is it that you will purchase this sample within the next two weeks?

1	2	3	4	5	6	7
Extremely unlikely	Very unlikely	Somewhat unlikely	Not sure	Somewhat likely	Very likely	Extremely likely

2. How likely is it that you will purchase this sample within the next month?

1	2	3	4	5	6	7
Extremely unlikely	Very unlikely	Somewhat unlikely	Not sure	Somewhat likely	Very likely	Extremely likely

3. How likely is it that you will buy this sample if available in supermarkets?

1	2	3	4	5	6	7
Extremely unlikely	Very unlikely	Somewhat unlikely	Not sure	Somewhat likely	Very likely	Extremely likely

4. How likely is it that you will use this sample as a substitute for spinach beet?

1	2	3	4	5	6	7
Extremely unlikely	Very unlikely	Somewhat unlikely	Not sure	Somewhat likely	Very likely	Extremely likely

5. How likely is it that you think you will get used to the taste of this sample?

1	2	3	4	5	6	7
Extremely unlikely	Very unlikely	Somewhat unlikely	Not sure	Somewhat likely	Very likely	Extremely likely

6. How much will you be willing to pay for a 300 g packet of fresh leaves of this sample?

1	2	3	4	5	6	7
R0	R1 to R5	R6 to R10	R11 to R15	R16 to R20	R21 to R25	R26 to R30

ANNEXURE 11

Summary of the sensory descriptors for spinach beet and lucerne samples cultivars used in this study.

	SBP	SBS	'SASP'	'SASS'	'WL525P'	'WL525S'	'WL711P'	'WL711S'
Aroma								
Grassy	X (H)	X (L)	X (H)**			X (L)*		
Spinach		X (H)		X (H)**			X (L)	
Onion						X (H)		X (L)
Potato		X (H)		X (L)		X (H)**		
Appearance								
Soft		X (H)		X (H)**			X (L)	
Wet		X (H)	X (L)	X (H)**				X (H)**
Fibrous		X (L)		X (L)*			X (H)	
Mushy		X (H)		X (H)**				X (L)
Mouthfeel								
Soft	X (H)		X (H)**		X (L)			
Chewy		X (L)		X (L)*			X (H)	
Fibrous		X (L)		X (L)*	X (H)		X (H)	
Mushy		X (H)		X (H)**				X (L)

L = Lowest value for all the samples; H = Highest value for all the samples; * = Lowest value for all the lucerne samples;

** = Highest value for all the lucerne samples.

SBP = spinach beet plain; SBS = spinach beet stew; 'SASP' = 'SA Standard plain'; 'SASS' = 'SA Standard stew';

'WL525P' = 'WL525 plain'; 'WL525S' = 'WL525 stew'; 'WL711P' = 'WL711 plain'; 'WL711S' = 'WL711 stew.'

ANNEXURE 11 continued

	SBP	SBS	'SASP'	'SASS'	'WL525P'	'WL525S'	'WL711P'	'WL711S'
Taste								
Salty		X (H)	X (L)	X (H)**				
Bitter		X (L)		X (L)*			X (H)	
Aftertaste								
Salty	X (L)		X (L)*		X (H)			
Bitter		X (L)		X (L)*			X (H)	
Metallic		X (L)	X (H)	X (L)*				

L = Lowest value for all the samples; H = Highest value for all the samples; * = Lowest value for all the lucerne samples;

** = Lowest value for all the lucerne samples.

SBP = spinach beet plain; SBS = spinach beet stew; 'SASP' = 'SA Standard plain'; 'SASS' = 'SA Standard stew';

'WL525P' = 'WL525 plain'; 'WL525S' = 'WL525 stew'; 'WL711P' = 'WL711 plain'; 'WL711S' = 'WL711 stew.'

ANNEXURE 12

KNOWLEDGE

Please complete the following by indicating with a X. No answers are considered incorrect, but honesty will be appreciated.

1. **Do you know what lucerne is?**

YES	1
NO	2

2. **If answered YES at QUESTION 1, please tell us what lucerne is, by indicating with a X at <u>each</u> statement:**

		YES	NO
1	A forage legume		
2	A plant		
3	Used for animal feed		
4	The capital of Switzerland		
5	Lucerne sprouts are sold in supermarkets		
6	Lucerne is used for animal feed		
7	Lucerne can be consumed by humans		
8	Lucerne is highly nutritious		
9	Lucerne is safe to eat		
10	I don't know		

3. **Do you know what alfalfa is?**

YES	1
NO	2

4. **If answered YES at QUESTION 3, please tell us what alfalfa is, by indicating with a X at <u>each</u> statement:**

		YES	NO
1	A forage legume		
2	A plant		
3	Used for animal feed		
4	It is the same as Lucerne		
5	Alfalfa sprouts are sold in supermarkets		
6	Alfalfa is used for animal feed		
7	Alfalfa can be consumed by humans		
8	Alfalfa is highly nutritious		
9	Alfalfa is safe to eat		
10	I don't know		

5. **Have you ever consumed lucerne (except for today)?**

YES	1
NO	2

6. **If answered YES at QUESTION 5, please answer the following, by indicating with a X at <u>each</u> statement:**

   a. **In which form did you consume lucerne?**

		YES	**NO**
1	Raw/Fresh		
2	Cooked		
3	Dried		

   b. **What part of lucerne have you consumed?**

		YES	**NO**
1	Leaves and stems		
2	Only the leaves		
3	Only the stems		

   c. **How often do you <u>eat</u> lucerne?**

	Eat
Everyday	1
Not every day, but 2-6 times a week	2
Once a week	3
Once every two weeks	4
Once every three weeks	5
Once a month	6
Les often	7
Never	8

   d. **How often do you <u>use</u> lucerne?**

	Use
Everyday	1
Not every day, but 2-6 times a week	2
Once a week	3
Once every two weeks	4
Once every three weeks	5
Once a month	6
Les often	7
Never	8

Thank you for your time.

ANNEXURE 13

ATTITUDES

Please complete the following by indicating with a X how much you disagree or agree with the following statements. No answers are considered incorrect, but honesty will be appreciated.

		Strongly disagree	Disagree	Neutral	Agree	Strongly agree
1	I reflect about my health a lot.					
2	I'm very conscious about my health.					
3	I'm alert to changes in my health.					
4	I'm usually aware of my health.					
5	I take responsibility for the state of my health.					
6	I'm aware of the state of my health as I go through the day.					
7	Nowadays most foods contain residues from chemical sprays and fertilizers.					
8	I'm very concerned about the amount of artificial additives and preservatives in food.					
9	The quality of and safety of vegetables nowadays concern me.					
10	I feel calm after eating lucerne.					
11	I think lucerne has market potential.					
12	I feel guilty after eating lucerne.					
13	I will recommend lucerne to other people.					
14	Lucerne is a natural product.					
15	Lucerne is very healthy for me.					
16	Lucerne has a pleasant taste.					
17	Lucerne has a pleasant smell.					
18	Lucerne has a pleasant mouth feel.					
19	Lucerne has a pleasant appearance.					
20	Lucerne has a bitter taste.					
21	Lucerne has a sour taste.					
22	Lucerne has a salty taste.					
23	Lucerne has a sweet taste.					

		Strongly disagree	Disagree	Neutral	Agree	Strongly agree
24	Overall I think that eating lucerne is beneficial.					
25	Overall I think that eating lucerne is pleasant.					
26	Overall I think that eating lucerne is the right thing to do.					
27	Overall I think that eating lucerne is good practice.					

Please complete the following by indicating with a X how much it is unlikely or likely that you agree with the following statements. No answers are considered incorrect, but honesty will be appreciated.

		Very unlikely	Unlikely	Neutral	Likely	Very likely
28	How likely is it that you will purchase lucerne produce when named lucerne on the label?					
29	How likely is it that you will purchase lucerne produce when named alfalfa on the label?					
30	If I eat lucerne, I will feel that I am doing something positive for my health and wellbeing.					
31	If I eat lucerne, I will feel that I add natural goodness to my meal.					
32	If I am aware of the benefits of lucerne, I will feel more comfortable to eat lucerne.					
33	If I eat lucerne, I will feel that I can regard lucerne as a type of leafy vegetable.					
34	If I eat lucerne, I feel that I can compare lucerne to spinach.					
35	If I can prepare lucerne in the same way as spinach, I will feel more comfortable to eat lucerne.					

Please complete the following by indicating with a X how much undesirable or desirable you find the following statements. No answers are considered incorrect, but honesty will be appreciated.

		Very undesirable	Undesirable	Neutral	Desirable	Very desirable
36	Experiencing a bitter taste is:					
37	Regarding lucerne as a leafy vegetable is:					
38	Comparing lucerne to spinach is:					

39. What do you believe are the advantages of eating lucerne?

____

____

____

____

40. What do you believe are the disadvantages of eating lucerne?

____

____

____

____

41. Please indicate if there is anything else you associate with eating lucerne?

____

____

____

____

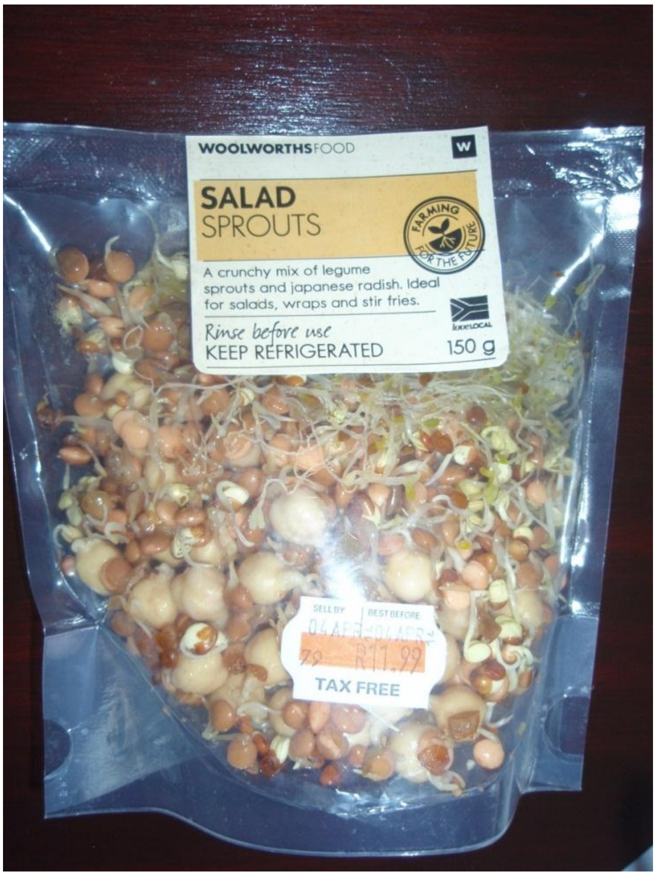
42. Are you interested to take part in a trained consumer panel to evaluate lucerne?

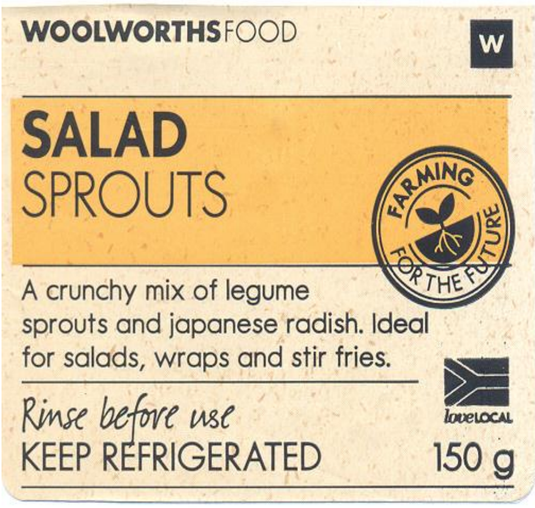
YES	1
NO	2

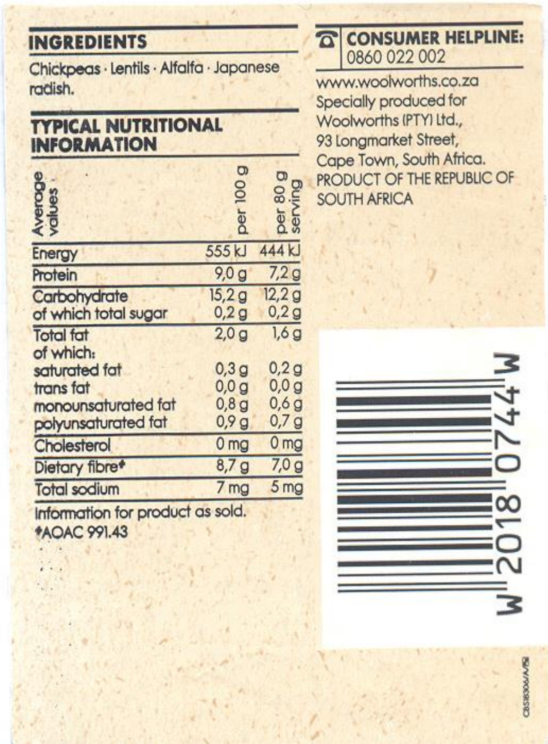
43. If answered YES, please report to the researcher(s) or assistant(s) in order to provide us with your contact details.

Thank you for your time.

ANNEXURE 14







Average values	per 100 g	per 80 g serving
Energy	555 kJ	444 kJ
Protein	9,0 g	7,2 g
Carbohydrate	15,2 g	12,2 g
of which total sugar	0,2 g	0,2 g
Total fat	2,0 g	1,6 g
of which:		
saturated fat	0,3 g	0,2 g
trans fat	0,0 g	0,0 g
monounsaturated fat	0,8 g	0,6 g
polyunsaturated fat	0,9 g	0,7 g
Cholesterol	0 mg	0 mg
Dietary fibre*	8,7 g	7,0 g
Total sodium	7 mg	5 mg

Information for product as sold.
*AOAC 991.43

ANNEXURE 15

Summary of participants' beliefs regarding the advantages of eating lucerne.

Category	Sub-category	Quote
Health	General	"It is good for your health."
		"With global warming taking place maybe this is a way in which more, healthy food is trying to be introduced in larger quantities."
		"It is good for the health because it is part of vegetables and it gives energy as well."
		"It is healthy and it is also a beneficial to your health."
		"It helps regarding my health issues."
		"I think it may play a vital part for healthy living and create a stable balance diet for a healthy life."
		"I think it is much healthier than spinach."
		"It is healthier than red meat."
	Body	"Making you strong and help to build your blood cells."
		"I believe it is healthy for your body."
		"Helps with coagulation of blood."
		"It boost your immune system and helps to fight against disease."
		"Clogging of blood."
		"Makes your skin healthier."
		"It boosts the body."
		"To get bones strong. Improve appetite."
Nutritious	General	"It's probably more nutritious than grains on the market and you will probably need less to get the same benefits with normal grains."
		"I do believe that it has a lot of nutritional value and can help consume vitamins and minerals necessary for healthy growth."
		"For our bodies we need vitamins so I think I will like to eat it more often."
		"It has some nutrients and vitamins that other leafy vegetables have."
		"I do believe that if lucerne is regarded as a leafy plant it ought to have similar nutrients as that of spinach, therefore good for the body."
		"High in vitamins and minerals."
		"Has higher nutritional value which spinach lacks."

Category	Sub-category	Quote
Nutritious	Iron	"Comparing it to spinach, it is very high in iron." "High iron content." "It's got iron (high in iron), which increases your iron levels in your blood preventing any sort of anaemia from occurring." "Has high iron which is good for people who want to donate blood."
	Protein	"It has high protein content." "I believe eating lucerne is healthy because it is rich in proteins so those who cannot take meat as a source of protein can replace it with lucerne." "It has high protein content as compared to other leafy vegetables." "Good source of protein if you can't afford other sources of protein."
	Fibre	"It contains good fibre." "It is high in dietary fibre." "Good roughage for your digestive health." "Giving you a "fullness" feeling." "A form of detoxing." "It is probably good for prevention of cancer and moderating the bowel movement since it seems to contain roughage.
Natural		"I believe it is a natural plant that is nutritious." “It is pure natural. No chemicals used that is healthy for us." "Naturally produced product." "Since it's a natural product it cannot be bad for you, especially if produced without harmful chemicals." "I think because it is natural it can give a person energy and boost some other parts of our body." "Does not contain artificial additives." "It has no additives and preservatives and most likely no artificial ruminants of chemicals." "I think it is a natural plant that I think would be healthy if used on daily basis."

Category	Sub-category	Quote
Vegetable	General	"They are good for health since it is a green vegetable." "Similar to the advantages of eating any other leafy vegetable." "Comply with the benefits of green leafy vegetables." "Green leafy vegetable." "It is healthy in terms of a person's diet because it is a vegetable." "Since it is green, one can gain the benefit like in other green vegetables."
	Spinach	"I believe it is like spinach and all the other vegetables which are good for my health." "It has the same goodness as spinach." "Replacer for spinach." "If prepared correctly, it could taste almost like spinach and could thus be used as spinach substitute." "I think it has similar benefits to spinach."
Sensory properties		"It has a good spinach aroma." "I think lucerne is very tasty." "It tastes good." "It is organic and it has a better taste than spinach. I would rather eat lucerne than spinach." "Eating lucerne is a good idea because it tastes like spinach."
Preparation		"Cook it in many different ways." "Easy to cook."
Costs		"It can be affordable." "More vegetables for people to eat and can be cheaper." "Getting in more greens at a cheaper price." "I think lucerne will be cheaper and it is good for your health." "It might be a cheaper alternative to spinach and will add variety to the vegetable market." "It's cheaper than meat."

Category	Sub-category	Quote
Availability		"It will increase food availability in the market and it is healthy." "It could be more widely available, which means one will not have to wait for it to be in season." "Made easy accessible to consumer."

Summary of participants' beliefs regarding the disadvantages of eating lucerne.

Category	Sub-category	Quote
Health risks	General	"You will get sick."
		"Pregnant woman may not eat."
		"It's not natural, could have side effects later on in life."
		"Is not yet proofed for human use."
		"I think it will make my stomach running."
		"May feel bloated."
		"Not sure if safe."
		"Not knowing what all the health advantages are."
	Allergies	"Other people are allergic to green legume seeds."
		"To cause allergies I associate with."
		"It might trigger some allergies for some people."
	Digestion	"I think for human consumption it will be difficult to digest."
		"It might not be easy for humans to digest it and break it up, thus health risk."
		"I tasted some products during the tasting that are hard to swallow, so I think there might be problems when it comes to indigestion."
	Chemicals	"It could contain unhealthy substances."
		"Artificial chemical sprays used at production."
		"Has added chemicals in."
		"Might have been produced by use of chemical fertilizers."
Nutritious		"Lucerne is of little nutritional value for humans."
		"Missing all the proteins and vitamins for your health."
		"That you will not reep the same benefits that you would from natural vegetables."
		"It could have less nutrients."
		"I think the fibre is way higher than the desired for human consumption."
		"It might contain cellulose."

Category	Sub-category	Quote
Sensory properties		"Sometimes it is bitter thus it will not be suitable for the whole family, especially children." "Some people may not like the bitterness." "Texture not always desirable." "The texture is rough and it gives a very parched feeling." "It doesn't have a smooth texture." "Has a distinctive smell associated with animal feed."
Positive feedback		"I believe that there are no disadvantages." "None because it is a nutritious plant and it is healthy to eat green vegetables." "Nothing as it is a natural product." "I honestly believe there aren't any disadvantages." "Lucerne is a leafy vegetable, there is none whatsoever negative thing about it." "I don't think it can have any major disadvantages." "Do not think there is any harm in eating lucerne." "No disadvantages only benefits."
Animal feed		"It is not meant for humans." "People view it as cattle feed." "Eating the same products as animals." "Less food for the animals." "Competition with animals." "Regarded as animal feed."
Preparation		"Much work to harvest and only leaves can be eaten." "How it is prepared it can change the taste." "They might be a bit unstable to heat." "Yes if not washed or prepared properly." "If it could be prepared in other ways it maybe be better and can be used more often."

Category	Sub-category	Quote
Cost		"Maybe expensive." "Animal feed prices might increase if humans start to compete with animals for the same feed sources." "Competing with livestock, will make lucerne expensive for farmers." "Livestock might pay more cause of the high demand." "More farmers will start to farm with lucerne and the price will decrease."
Frequency of consumption		"Just don’t eat too much or excessively. Eating moderately." "If you eat it every day." "I think you will quickly get tired of it if you eat too much of it." "Too much consumption may lead to toxicity." "If a person does not consumed too much of it but moderates it."

Summary of participants' associations regarding the eating of lucerne.

Category	Sub-category	Quote
Health		"It will probably reduce risks of getting ill." "Increasing vitamin A intake." "It is healthy." "Lucerne seems like it has antioxidants which purify or clean the body." "I see it as a good thing seeing that the market is now full of products that are not natural." "It's good practice for healthy eating. Good food for the old." "It can be purchased for medicine usage." "People say lucerne is good for high blood."
Vegetables	Spinach	"Spinach, it resembles it, and does somewhat taste like it." "Lucerne is tasty and nutritious, hence worth being associated with spinach." "I associate it with eating green vegetables like spinach." "Taste the same as spinach." "Spinach you find growing in the wild." "Lucerne is the same as spinach."
	Other vegetables	"It tastes like morogo." "It's like eating spinach and cabbage (leafy vegetables)." "Squash before adding sugar." "Potatoes mashed. Carrots mashed." "Wild leafy vegetables." "Traditional leafy vegetables." "Traditional morogo." "Clover." "I associate it with mushrooms." "Cabbage leafs." "Green beans." "Gem squash and it tastes like pumpkin and baby marrows." "Amaranthus."

Category	Sub-category	Quote
Serving suggestions		"Eating it with meat or having it in a quiche." "Porridge." "Maize meal." "Meat will help with its saltiness." "Try more potatoes, black pepper and Worcester sauce." "I will associate cheese with lucerne and a white sauce." "The texture needs to be improved, make it firm." "It may be eaten with porridge. Mix it with spinach and some spices." "Maybe put salt or butter in, it will be tasty." "Potatoes with butter and white pepper."
Sensory properties		“It was like eating grass.” “Lucerne tastes like it can help you clear out your system.” “With some more added rich natural taste, which make lucerne more special.” “It has taste.” “Texture is essential to me.”

ANNEXURE 16

SUGGESTED LUCERNE RECIPES

1. Lucerne stew:

Ingredients:

250 ml lucerne leaves

2 tablespoons olive oil

1 chopped onion

1 large potato

2 carrots

250 ml vegetable/chicken broth

250 ml white/brown rice

1 clove garlic

Salt and pepper

Preparation:

Cook rice for 20 minutes.

Chop potato, carrots, garlic and onion and sauté in the oil.

Add rice and lucerne leaves.

Add vegetable broth or water and boil for 20 minutes.

Add salt and pepper if preferred.

2. Lucerne soup:

Ingredients:

250 ml lucerne leaves

250 ml vegetable/chicken broth

10 g flour

10 g butter

100 ml milk

125 ml cream

Preparation:

Cook lucerne leaves in boiling water for 10 minutes and drain.

Put butter in a saucepan; melt over moderate heat; add flour and cream to form a fairly thick paste; cool.

Add boiling broth very slowly; stir constantly to avoid lumps. Return to the stove and when sauce begins to boil, add the lucerne.

Boil 20 minutes, stirring occasionally. Add milk to desired consistency.

If a velouté soup is desired, add one-half beaten egg yolk to above proportions.

Serve with choice of bread if preferred.

3. Lucerne savoury muffins:

Ingredients:

250 ml lucerne leaves

125 ml oil

200 ml water

250 g savoury muffin mix

Preparation:

Cook lucerne leaves in boiling water for 10 minutes and drain.

Preheat oven to 180°C.

Place premix into mixing bowl.

Lightly beat water, oil and eggs and add to premix.

Mix with a spoon until just combined.

Add cooked lucerne leaves to premix and do not over mix.

Spoon equal quantities of the mixture into a well-greased muffin pan (two-thirds full).

Bake for 25 minutes until golden brown.

Serve with cheese and butter if preferred.

4. Maize porridge with lucerne:

Ingredients:

250 ml lucerne leaves

250 ml maize meal

500 ml water

¼ teaspoon salt

Preparation:

Cook lucerne leaves in boiling water for 10 minutes and drain.

Bring water and salt to the boil in a large pot.

Add maize meal. Stir thoroughly with two-pronged fork and cover with lid.

Reduce heat and simmer for 40 minutes and stir occasionally.

Add 60 ml cold water to porridge.

Simmer over low heat for another 30 minutes.

Mix porridge with lucerne leaves and serve.