THE MORPHOLOGICAL, PHYSIOLOGICAL, PHYSICO-CHEMICAL EVALUATION AND SENSORY PROFILING OF NOPALITOS FROM 20 SOUTH AFRICAN CACTUS PEAR CULTIVARS

By

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Declaration

I declare that the dissertation hereby handed in for the qualification of M.Sc. Food Science at the University of the Free State, is my own independent work and that I have not previously submitted the same work for a qualification at/in another university/faculty.

I hereby concede copyright of this dissertation to the University of the Free State.

…………………………………………………………………
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Ke na le Modisa, Ke tla be ke hlokang (I have a Shepard, I shall not want).

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Table of Contents

List of Tables viii

List of figures xi

Summary/Abstract xiii

Chapter 1 1

1.1. Introduction 1
1.2. Aims 2

Chapter 2 4

2. Literature review 4
2.1. History of the cactus pear 4
2.2. Introduction of cactus pear plants to South Africa 5
2.3. Nopalitos 6
2.4. Consumer analysis 22
2.5. Conclusion 23

Chapter 3 24

3. The morphological, physiological and physico-chemical evaluation of nopalitos from twenty South African cactus pear cultivars 24
3.1. Introduction 24
3.2. Materials and methods 25
3.3. Statistical analysis 29
3.4. Results and discussion 29
3.5. Summary 76
3.6. Conclusion 78

Chapter 4 80

4. Sensory profiling of nopalitos from 20 cactus pear cultivars, using the Check-All-That-Apply (CATA) technique 80
4.1. Introduction 80
4.2. Material and methods 82
4.3. Results and discussion 86
4.4. Conclusion 98

Chapter 5 99

5. The nutritional evaluation of nopalitos from six South African cactus pear cultivars 99
5.1. Introduction 99
5.2. Materials and methods 100
5.2.1. Sample collection 100
5.2.2. Crude protein 100
5.2.3. Fibre 100
5.2.4. Fat analysis 103
5.2.5. Mineral analysis 104
5.2.6. Sucrose/D-Glucose/D-Fructose analysis 105
5.2.7. Starch analysis 107
5.2.8. Phytochemicals analysis 108
5.2.9. Antioxidant activity analysis 110
5.2.10. Statistical analyses 110
5.3. Results and discussion 110
5.3.1. Crude protein 113
5.3.2. Fibre 113
5.3.3. Fat analysis 118
5.3.4. Mineral analysis 127
5.3.5. Sucrose/D-Glucose/D-Fructose analysis 129
5.3.6. Starch analysis 130
5.3.7. Phytochemicals analysis 132
5.3.8. Antioxidant activity 134
5.4. Conclusion 136
Chapter 6 139
6.1. General discussions and conclusions 139
6.2. Recommendations for further study 142
Bibliography 143

List of Tables

Table 1: The chemical composition of cladodes from the Opuntia species whose spines have been removed ……………………………………………………………………………………11

Table 2: The organic acid composition of cladodes from the Opuntia species harvested at different times of the day ……………………………………………………………………12

Table 3: The predominant phenolic compounds found in cactus stems …………………..13

Table 4: Food and sub products from cactus pear fruits and stems ……………………..16

Table 5: Analysis of variance (ANOVA) for the influence of cultivar, year of harvest, as well as the cultivar and year of harvest interaction on the various nopalito attributes ……………………………………………………………………………………………31

Table 6: Analysis of variance (ANOVA) for the influence of cultivar morphological and physiological attributes of nopalitos harvested over two years ………33

Table 7: Analysis of variance (ANOVA) for the effect of cultivar on the various physical attributes of nopalitos harvested over two years ……………………………………38

Table 8: Analysis of variance (ANOVA) for the effect of cultivar on the various colour attributes of nopalitos harvested over two years ………………………………………..40

Table 9: Analysis of variance (ANOVA) for the effect of cultivar on the various physico-chemical and chemical attributes of nopalitos harvested over two years ……..44

Table 10: Analysis of variance (ANOVA) for the influence of year of harvest on the morphological and physiological attributes of nopalitos …………………………….49

Table 11: Analysis of variance (ANOVA) for the influence of year of harvest on the physical attributes of nopalitos ………………………………………………………………50

Table 12: Analysis of variance (ANOVA) for the influence of year of harvest on the physico-chemical and chemical attributes of nopalitos ………………………………53

Table 13: Analysis of variance (ANOVA) for the effect of cultivar and year harvest interaction on the morphological attributes of nopalitos ....56 - 57

Table 14: Analysis of variance (ANOVA) for the effect of cultivar and year harvest interaction on the yield of pulp and mucilage data from the 20 nopalito cultivars ....58 - 59

Table 15: Analysis of variance (ANOVA) for the effect of the interaction between cultivar and the year of harvest on the colour properties of nopalitos ....64 - 65

Table 16: Analysis of variance (ANOVA) for the effect of cultivar and the year of harvest interaction on the physical properties of nopalitos ....68 - 69

Table 17: Analysis of variance (ANOVA) for the effect of cultivar and the year of harvest interaction on the physico-chemical and chemical properties of nopalitos ....72 - 73

Table 18: A summary of the best year of harvest for nopalito attributes 80

Table 19: Demographic profile and cluster analysis of the 61-member consumer panel, for nopalitos from 20 cactus pear cultivars, cucumber and green pepper 85

Table 20: Analysis of variance for consumers’ overall liking of nopalito samples, from 20 cultivars of cactus pears and two controls, namely cucumber and green pepper 88

Table 21: Summary of sensory characteristics, frequency of use and significant differences between genotypes for the CATA question ....89 - 90

Table 22: Analysis of variance (ANOVA) for the effect of cultivar on the various nutritional properties of nopalitos ……….……………………………..…………………….112 - 113

Table 23: The fatty acid composition of the six nopalito cultivars ……………………………117 - 118

Table 24: An indication of the highest nutritional contents found among the six nopalito cultivars that were investigated in this study …………………………………………..137 - 139

List of figures

Figure 1: Schematic representation of the workflow of this study ……………..…………..….3

Figure 2: The coat of arms of Mexico …………………..……………………………………………………..4

Figure 3: The CAM cycle …………………..………………………………………..……………………………..8

Figure 4: Growth stages of cactus stems ………………..……………………………………..………….9

Figure 5: Glochids and spines on a cladode …………..…………………………………………………10

Figure 6: L*a*b colour space chromaticity diagram ……………………………………………….43

Figure 7: The effect of the year of harvest on the extraction of mucilage parameters obtained from the various nopalito cultivars over the two years ………………..……………………………………………………..…………………………………50

Figure 8: The processing of various harvested nopalito cultivars ……………………………83

Figure 9: A foam tray filled with various three-digit coded samples for the lexico test as well as the CATA sensory analysis………………………………………………………..….84

Figure 10: CATA analysis in progress ……………………………………………………..……………86

Figure 11: Sample and term representations in the first and second coordinates of the correspondence analysis, performed on the frequency of the CATA questions for the evaluation of nopalitos, from different cactus pear cultivar……….…..95

Figure 12: Dendrogram of the AHC, indicating the presence of four clusters of consumer's overall hedonic acceptability of different nopalitos from different cactus pear cultivars ………………………………………………………………………………………………97

Figure 13: External preference map of identified consumer clusters, of nopalitos from 20 different cultivars, as well as cucumber and green pepper ………………………...98

Figure 14: Various nopalito samples being investigated for Acid Detergent Fibre content ……………………………………………………………………………………………………….…..102

Figure 15: Extraction of total lipid content in various nopalito cultivars in progress …………………………………………………………………………..…………………………………104

Figure 16: Transesterification of various nopalito cultivars in progress ……………………….105

Figure 17: Preparation of samples for sugar and starch analyses ….……………………………109

Figure 18: The resulting starch sugar interconversion found in the 6 different nopalito cultivars ………………..………………………………………………………………………………………132

Summary/Abstract

The exponential increase of food insecurity around the world warrants for the development of mitigation strategies which include looking for new food sources that will not only minimise the effects of food insecurity but are also sustainable. The cactus *Opuntia* produces edible stems (cladodes) and fruits with high nutritional value in terms of minerals, protein, dietary fibre and phytochemicals, and with the increase in the global shift towards the use of natural plant materials in various industries including food, cosmetics as well as pharmaceuticals, the cactus pear plant makes for great interest. In addition to its multifaceted properties, the plant is appealing all the more in the South African landscape because of its high ecological adaptivity, with most of its species being able to survive in arid and semi-arid places. As such, a comprehensive study on the young edible stems (nopalitos) from twenty South African cactus pear cultivars was conducted because they are the one part of the cactus plant that is underutilised and consequently almost exclusively used for animal feed.

Nopalitos from the twenty South African cultivars were harvested and various morphological, physiological, physico-chemical and chemical tests were conducted over 2 years (2018 and 2019). These tests included the weight, moisture content, solids, sugar content, acidity, colour, mucilage content and viscosity thereof, and pH which are some of the main factors which affect the palatability and overall functionality of the vegetable. Further investigations included the nutritional content of the stems as well as the sensorial attributes to determine the overall eating quality and acceptability of the stems.

Upon concluding the various experiments, interesting trends were noticed between the two years with the 2018 harvest having produced more favourable attributes than the 2019 harvest. The 2018 harvest produced nopalitos which were thinner and greener stems which had the least mucilage content and viscosity while presenting high values of solids and sugar. These attributes are important as they affect the taste, keeping qualities and overall acceptability of the samples the most.

When comparing the effects of cultivar, year of harvest and the effects of cultivar and year of harvest interaction on the various nopalito attributes, it was observed that the effect of cultivar on the various parameters had the most significant differences. These significant differences were found in the weight (g), length (cm), width (cm), diameter (cm), pulp (before centrifuge) (g), extracted mucilage (g), waste (g), % mucilage yield, firmness (mm), titratable acidity expressed as % citric acid, line-spread (cm) method of determining viscosity, sugar (TSS °Brix) content as well as the colour coordinates of the various nopalito cultivars (L*, a*, b*, Chroma (Saturation Index) and hue angle). The best overall cultivars following this investigation were Meyers, Malta, Nudosa, Fusicaulis, Fresno and Morado.

Sensory profiling of the nopalitos from twenty South African cactus pear cultivars aimed to determine consumer acceptability showed interesting variations. The cultivars were easily comparable with green pepper and cucumber in certain aspects. Among the twenty nopalito cultivars, Skinners Court, Turpin, Fusicaulis, R1251 and Rossa were 'neither liked nor disliked' by the consumers, as they were all ranked higher than the other cultivars, while Robusta was the least liked cultivar.

Nutritional analyses were conducted for the six nopalito cultivars which were deemed to be the overall best following the morphological and physico-chemical analyses as well as the consideration of the sensorial properties of the different cultivars. From this investigation, Malta, Fusicaulis and Meyers were deemed as the best cultivars out of the six as they presented the highest nutritional content.

Overall, the reported work has shown the high quality of the nopalitos as a vegetable source which is easily and competitively compared to other known vegetables/crops such as green pepper and cucumber in terms of sensorial properties and oranges, soybeans and other nopalito cultivars found in various parts of the world in terms of nutritional, morphological and physico-chemical properties.

It is recommended that future research into the nopalitos include ways in which the stems can be prepared and incorporated into different products to increase the overall acceptability of South African consumers.

Key terms: Food security**,** *Opuntia ficus-indica*, cactus pear, nopalitos, cultivar, morphological properties, physico-chemical properties, nutritional content, Check-All-That-Apply, consumer acceptability, South Africa.

Chapter 1

1.1. Introduction

The impacts of the economic meltdown, climate change as well as the existence of the ever-increasing strain on agricultural systems have raised to the fore, issues related to food and nutrition security. In fact, food insecurity has been recognised as a global crisis (USAID, 2009). According to the Food and Agricultural Organization (2004), it is estimated that more than 814 million people located in developing countries are undernourished, of which 204 million of these people live in sub-Saharan Africa, which includes South Africa.

In the South African context, poverty and unemployment have been deemed as some of the biggest calamities which have often been exacerbated by the ever-increasing cost of living (United Nations, 2009). Consequently, these have added additional pressure to many households as they continuously struggle to make ends meet. One of the most immediate aspects of life in which these cascading effects ripple through is the quality of the population's daily dietary regime.

The United States Department of Agriculture (USDA, 2011) defines food security as the ability of all members of a society to have enough food in order to sustain their daily lives. In essence, this translates to the availability of nutritious and safe food as well as a steady means to access and purchase these foods in an effective manner (Labadarios *et al.,* 2011). On the other side of the spectrum, food insecurity involves the limited and/or inability of accessibility to food sources.

The combination of the abovementioned factors which contribute to the food insecurity of South Africa, warrants for the consideration and investigation into alternative sustainable food sources that will not only be nutritious, but also alleviate the existing strain on agricultural systems as well as many of the households' pockets. One such alternative source is the nopalitos which are the young edible stems from the *Opuntia* species of cactus pear plants. The cactus pear plant has been cultivated for its fruits, flowers, seeds and stems for both humans and animals for many years in various parts of the world, though Mexico has been reported to being the place of origin (Rodriguez-Felix, 2002). This species of plants are known to grow in various agricultural and climatic conditions. This can be attributed mainly to their particular photosynthetic metabolism which allows the plants to retain as much water as possible, therefore being able to survive and thrive under the harshest of conditions (Stintzing & Carle, 2005).

The modified leaves (nopalitos) of the cactus pear plant are flat, thin, and oval and have been consumed as a staple vegetable in Mexican cuisine for several years. The versatility of their preparation can be seen in raw dishes such as salsas and salads or being added to dishes such as beverages, desserts and sauces. Their consumption has been associated with several positive health and physiological effects (Peña-Valdivia *et al.,* 2008). For example, the advantages of their soluble, insoluble and total dietary fibre upon consumption have been reported. Furthermore, they have been reported to render protection against some diseases such as atherosclerosis, obesity, gastric ulcer and colon cancer, to name a few (Wolfram *et al.,* 2002; Galati *et al*., 2003; Corrales-García *et al*., 2004). More of their positive effects have been well-documented in the pharmaceutical and cosmetic industries where they serve different purposes (Felker & Inglese, 2003). For example, in the pharmaceutical industry, their pharmacological profile which includes antioxidant capacity, anti-inflammatory properties as well as anti-hyperlipidemic and cholesterol-lowering effects, have rendered their use in the treatment of several ailments (Stintzing & Carle, 2005).

With all of the benefits mentioned above, it is clear that the incorporation of these young stems into one's daily diet presents many health advantages. Be that as it may, the stems still have not found their way into the South African commercial sector as a vegetable, as more often than not, they are processed and sold as forage to livestock farmers (Potgieter & Smith, 2006; De Waal *et al.,* 2015). This provides an opportunity to conduct extensive research which will advocate for its introduction into the South African agricultural sector as a commercial vegetable. This is not only important for developing a sustainable food source, but also to reduce the impacts that food insecurity has had on several livelihoods.

1.2. Aims

The aims of this study were as follows:

a. To thoroughly investigate the history of the cactus pear plant, the functional use of its various parts with a much deeper analysis of the 20 South African cultivars of young edible stems' properties which include the morphology, physiological traits, physical traits, physico-chemical traits, chemical traits as well as nutritional and sensorial properties. Because the mandate is to introduce these stems as a practical vegetable in South African markets, they were pre-selected from a total of 42 available cultivars as they presented the most favourable eating qualities.
b. To investigate the effects on the 20 cultivars' attributes brought about by the different cultivars, the year of harvest (2018 and 2019) as well as the interaction between the particular cultivars and the year of harvest.
c. To compare the 20 cultivars with common vegetables which they have been associated with in terms of eating qualities. These common vegetables are green pepper, cucumber, green beans and baby marrow.

d. To determine the best overall cultivar out of the six which were identified during sensory profiling as possessing the most potential as a staple vegetable in the country and should be immediately considered for commercialisation in South African markets.

In order to show the progress of the work that was done in this study, a schematic diagram is shown in Figure 1.

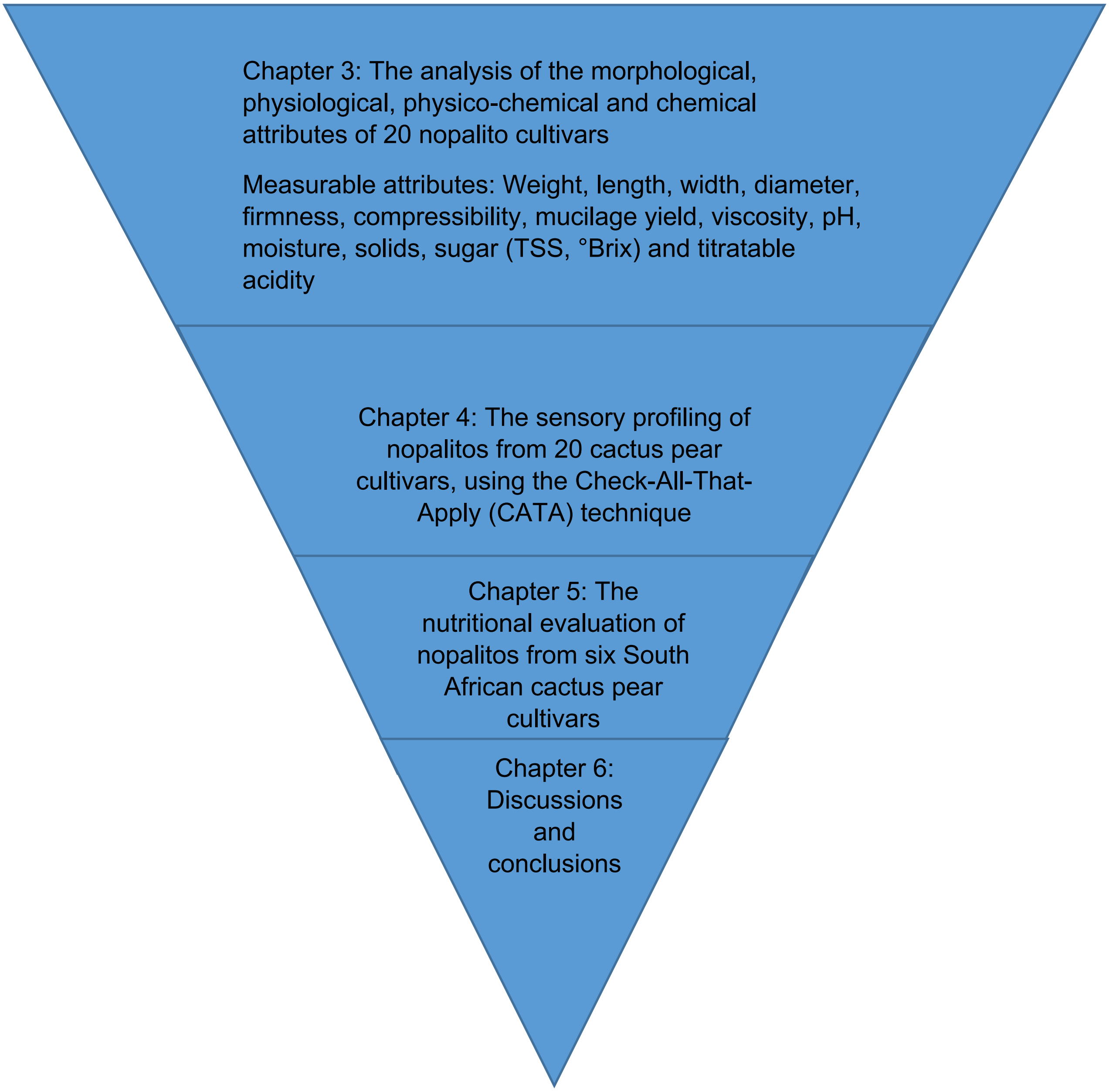

Figure 1: Schematic representation of the workflow of this study

Chapter 2

2. Literature review

2.1. History of the cactus pear

The *Opuntia* genera of prickly pears also known as cactus pear plants boasts a rich history which spans over 8000 years (www.dezeewierwinkel.nl, 2015). The plant's early historic presence can be traced back to as early as 1492 wherein natives of Hispaniola Island (presently known as the Dominican Republic and Haiti) introduced its fruit to the Spaniards (Ochoa and Barbera, 2017). The plant has since then formed an important part of the economy, culture and ecology of the areas which it inhabits. One such example is in the Aztec civilization whereby the plant signified the Aztecs' strength and dynamism. The level of importance of this plant to the Aztecs can still be observed in the present day in the coat of arms of Mexico (www.en.wikipedia.org, 2007) which depicts an eagle settled on a cactus plant while feeding on a snake (Figure 2). The introduction of the plant to the Spaniards translated to it being introduced to the rest of the world as it became adorned for its edible fruits, distinctive morphological and anti-scurvy properties as well as its symbiotic relationship with the scarlet dye-producing cochineal insect (www.dezeewierwinkel.nl, 2015).

Figure 2: The coat of arms of Mexico (www.en.wikipedia.org, 2007)

In its new territories, the plant found use in various ways other than fruit, vegetable and dye production. For example, Oviedo y Valdés, Toribio de Motolinia and Galeotto Cei (1539-1553) as cited by Ochoa & Barbera (2018), have all documented the plant's use as a herb, water source, barrier as well as a fence.

Furthermore, it was well-received as decoration in the many gardens of European countries (Ochoa & Barbera, 2018). Such examples of the plant's multifaceted nature have since caught the attention of communities and industries.

2.2. Introduction of cactus pear plants to South Africa

The deportation of the Moors back to their homelands from Spain (Diguet, 1928), as well as the Spanish expansion (Ochoa & Barbera, 2018), opened umpteen opportunities for cactus plantations in Africa. These countries include Ethiopia, Morocco and South Africa. The Cape was the first area in South Africa wherein the *Opuntia ficus-indica* species of the cactus plant was inhabited.

In the Cape of Good Hope, the cactus pear plant was cultivated for the fruit as well as for the purpose of serving as living fences. Over the coming 150 years from the time they were first introduced, the plants gradually returned to their spiny form (Ochoa & Barbera, 2018). This resulted in an infestation of impenetrable thickets in various regions, mainly in the Eastern Cape (Beinart & Wotshela, 2011). The cacti invasion was estimated at over 2 million hectares in the early twentieth century in South Africa alone, which heavily impacted the agricultural sector negatively. In an effort to combat the invasion, biological control using the cochineal insect, *Dactylopius ceylonicus* (Green) (Hemiptera: Dactylopiidae), was employed which salvaged approximately 80% of the infested plants (Zimmermann *et al.*, 2009).

Aside from the plants that have been reported in South Africa as early as 1772, 22 spineless cultivars were imported in 1914 from the Burbank Nursery in the United States of America (Agriorbit, 2019). Karoo farmers established these spineless cactus pear plants (*Opuntia ficus-indica* and *Opuntia robusta*) to feed their livestock so as to mitigate the drought that was present. Spineless cladodes have since proven to be the most effective in that their utilisation comes with reduced labour and waste.

There are currently 40 *Opuntia ficus-indica* cultivars and two *O. robusta* cultivars in Germplasm banks which can be found in the Limpopo, Free State, Eastern and Western Cape provinces (Ochoa & Barbera, 2018). The Mara germplasm in Limpopo has been observed to be the largest collection in South Africa and a complete "Burbank" collection in the world (Ledwaba *et al.,* 2012). The local and export potential of the cactus pear plant as a commercial fruit crop has risen over the past 15 – 20 years (Ledwaba *et al.,* 2012). The most popular cactus pear varieties in South Africa in relation to consumption are Morado, Gymno Carpo, Algerian and Skinners Court (Ledwaba *et al.,* 2012). All of these cultivars vary in terms of colour and the month of availability. For example, the Algerian cultivar produces exotic fruit that has a pink colour and is available in the middle of December; Gymno Carpo is yellow and is available at the beginning of December; Morado produces delicate white fruit, while

Skinners Court produces large sweet fruit which are green/white and are available in the middle of January (Ledwaba *et al.,* 2012).

These banks were established for research purposes which previously focused mainly on the use of the plant as forage (Menezes *et al*., 2010). For example, one study suggested Nepgen, Roedtan and Morado cultivars of cladodes to be used as fodder during seasons of drought due to their high mean number of cladodes that were observed after pruning (Mokoboki *et al.,* 2016). With the increasing interest in the plant's compatibility with human consumption and animal feed, particularly the young vegetable stems (nopalitos), the research focus has since shifted to human use.

2.3. Nopalitos

Nopalitos are the young, succulent and soft stems of the cactus plant which are mainly eaten as vegetables. These stems are from various *Platyopuntia* species mainly *O. ficus-indica* Mill., *O. ficus indica* var. *inermis* De candolle, *O. robusta* Wendl., *O. streptacantha* Lem., *O. amyclaea* Ten., as well as *Nopalea conchenillifera* (L.) (Mizrahi *et al*., 1997). Nopalitos have formed a large part of the Mexican culture and horticultural industry for several centuries. It is speculated that they have been utilised as early as the Mesoamerican civilisation where they often substituted the cactus pear fruit as a water source (Mondragón-Jacobo & Méndez-Gallegos, 2017). Furthermore, the cactus stem was regarded as the 'plant of life' because of its ability to produce new plants following a state of senescence. Presently in the domiciliary markets, the stems' consumption as a vegetable averages approximately 6.4 kg per capita and there is an increasing demand for them in Canada and the US (Ventura-Aguilar *et al.,* 2017). They are generally marketed in one of three ways: i) fresh nopalitos with thorns; ii) dethorned nopalitos (diced or whole) or iii) pre-cooked and frozen (Ventura-Aguilar *et al*., 2017). Mondragón-Jacobo and Méndez-Gallegos (2017) have noted that "the introduction of vegetable nopalitos in other countries and cultures has not been easy, despite the plant's adaptability and high productivity in most locations. The presence of thorns − even the so-called "spineless" cultivars present spines when young − the abundance of mucilage, and the lack of organoleptic appeal of cooked nopalitos have limited their acceptance".

Despite the aforementioned challenge, nopalitos are still highly appreciated for their medicinal and nutritional properties. These properties pose several opportunities for the stems' use in various industries; however little is still known regarding the processes behind their multifacetedness.

2.3.1. Morphology

The *Opuntia ficus-indica* species of cacti have evolved over the years to such an extent that they are able to thrive in adverse environmental conditions and water stress. This can be ascribed to the plant's anatomical, morphological and physiological traits which are evident in various parts of the plant such as the reproductive structures, roots as well as shoots (Prat & Franck, 2018). Pursuant to the literature, the plant's survival mechanism can be ascribed to its carbon uptake and water loss via CAM (Crassulacean Acid Metabolism (Prat & Franck, 2018). According to Inglese *et al*. (2018), the cycle is characterised by the following:

a. The temporary separation of carbon dioxide uptake and decarboxylation
b. The reduction of transpiration due to stomatal opening in the evening
c. Decreased water loss
d. Depressed root: shoot ratios and rapid growth of the root in wet conditions
e. The development of suberin in cortical cells combined with the forming of root-soil gaps during soil drying
f. The internal recycling of water from the parenchyma versus the chlorenchyma
g. The use of the phloem in order to furnish water and solutes to the fruit

Inglese *et al*. (2018) further summarised the CAM pathway (Figure 3) as follows:

a. In the first phase which takes place in the evening, the stomata opens up resulting in carbon dioxide fixation. Carbon dioxide diffuses into the mesophyllic intracellular spaces and then spreads into the cytosol, where it is bound to phosphoenolpyruvate (PEP), via PEP carboxylase (PEPc). The formation of oxaloacetate is catalysed by this enzyme which can be transformed into malate by NAD+ (nicotinamide adenine dinucleotide) malate dehydrogenase. Malate is actively transferred to the vacuole from the cytosol, where it is transfigured into malic acid; this occurs in an effort to avoid inhibition. An increase in acidity can be observed after this process takes place. The nightly buildup of organic acids results in the vacuoles of the cells of the chlorenchyma inhabiting more than 90% of the cell volume.
b. In the second phase, the PEPc transitions to Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) activity at dawn.
c. Phase 3 takes place diurnally, where plants' guard cells and stomata close and the pH rises. Malate disperses out of the vacuole and is decarboxylated. Carbon dioxide rises from 0.2 to 2.5% as it is released in the cytosol and fixed by Rubisco into the Calvin cycle. This results in starch synthesis or the synthesis of other glucans.
d. The activation of PEPc in the late afternoon signals the start of the final phase. This often becomes the only phase of the cycle in unfavourable (arid) environmental conditions.

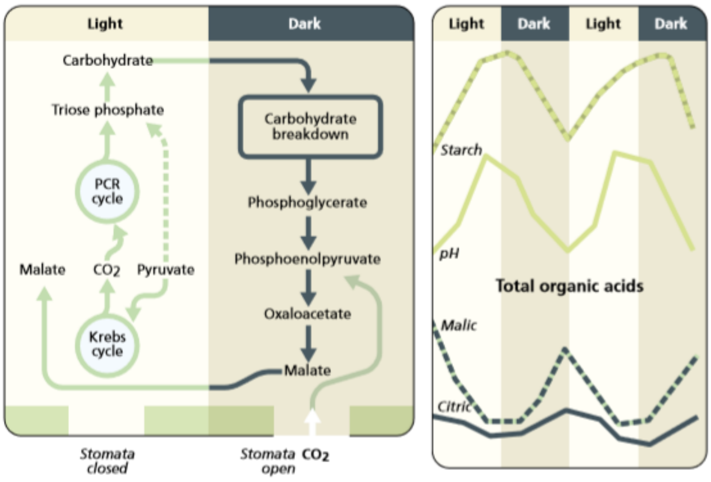

Figure 3: The CAM cycle (Inglese et al., 2018)

In addition to the CAM, the cactus stems consist of water-conducting tissue which has 4-9 xylem repositories per millimetre square that remain stable in various climates (Ventura-Aguilar *et al.,* 2017).

According to Prat and Franck (2018), the stems are typically oblong to spatulate-oblong in shape; range from 30-40 cm in length but can reach 70-80 cm and are 18-25 cm wide. Looking at the various growth stages of cactus stems (Figure 4), they are normally consumed when they have reached a length of 12 cm-20 cm and are marketed once they have reached 25cm in length (Ventura-Aguilar *et al.,* 2017). When cut in half, the stem mirrors an eustele, made up of cortex, pith, skin, and vascular tissue, in a ring and made from parallel bundles sundered by parenchyma tissue (Prat & Franck, 2018).

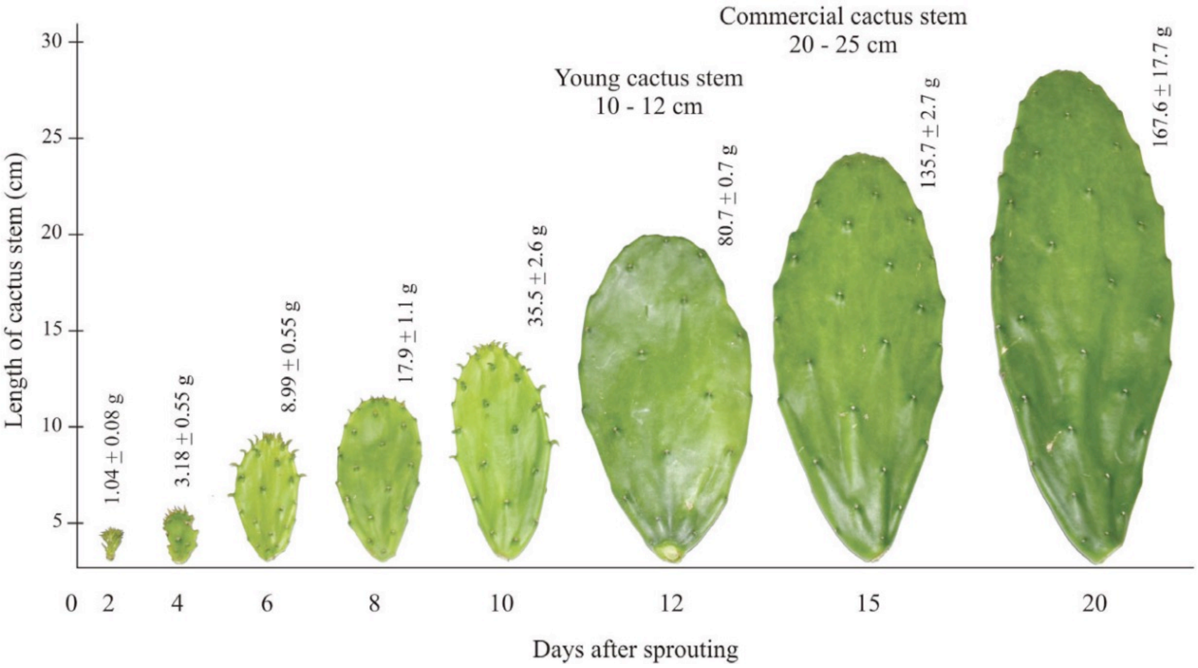

Figure 4: Growth stages of cactus stems (Ventura-Aguilar et al., 2017)

The skin of the stems consists of 6-7 layers of hypodermal cells (Prat & Franck, 2018) and a mono-stratified layer of epidermal cells (Ventura-Aguilar *et al*., 2017) which act as a barrier in preventing physical injuries and maintain the mechanical integrity of the stems (Prat & Franck, 2018). The skin is eventually substituted by the periderm (bark) which forms as a result of either the natural ageing process or injury in deeper tissues of the cortex when injured.

In addition to the epidermis bearing the stomata, Prat and Franck (2018) noted the layer as having three main functions: i) control the inflow of carbon dioxide and outflow of oxygen in the plant; ii) ensuring that water is retained within the plant and iii) shield the plant from fungi, insects and harsh sunlight (Mauseth, 1984).

Cutin is a fatty and waxy substance that covers the outermost wall of the epidermis and is responsible for the stems' glaucous appearance (Prat & Franck, 2018). Furthermore, this substance is one of the most natural chemicals known to man and is resistant to water (Mauseth, 1984). When the surface of *O. ficus-indica* stems was examined using scanning and transmission electron microscopy, a slender mass coating of a basic wax structure of semi-vertical plates was observed (Prat & Franck, 2018) which corresponds with the plates and scales classified in the basic group III of waxes (Metcalf and Chalk, 1979). The cuticle's waxy surface inhibits surface water and the escape of water vapour from the plants (Prat & Franck, 2018). With regards to the white cuticle, Gibson and Nobel (1986) have noted that its ability to reflect solar radiation ensures that the stem temperature is decreased. Mauseth (2006a) has noted that the epidermis in the majority of cacti is able to facilitate gas exchange unlike that of most seed plants.

O. ficus-indica cactus stems have low sunken stomatal density which is approximately $20mm^2$ (Ventura-Aguilar *et al*., 2017). The stomata that are present are randomly spread (Prat & Franck, 2018) on the epidermis stem and ensure reduced water loss as a result of transpiration (Ventura-Aguilar *et al*., 2017). Surrounding the stomata are druses which protect the stems against insects (Ventura-Aguilar *et al*., 2017).

Areoles on cactus stems are regarded as immensely specialised branches that give rise to glochids and spines which protect the stems (www.en.wikipedia.org/wiki/Areole, 2019). They further give rise to flowers (Jiménez-Sierra & Reyes, 2000). The areoles are oval in shape and are located under the surface of the skin (Prat & Franck, 2018).

Glochids and spines (Figure 5) are morphologically considered as the leaves of the cactus stems (Boke, 1944). It is easy to distinguish between the two as a result of their varying differences. For one, glochids are known to have a smooth surface while spines have a rough one (Robinson, 1974). Glochids comprise 100% crystalline cellulose (Anderson, 2001) while spines are composed of 96% polysaccharides (particularly arabinan (50.3%) and cellulose

(49.7%) as well as ash, fat, lignin and waxes (Ventura-Aguilar *et al*., 2017). According to Gamong, as cited by Buxbaum (1950), spines are arranged in series while glochids are formed in 7-12 clusters (Prat & Franck, 2018). Furthermore, glochids are known to be brown in colour while the spines are white (Prat & Franck, 2018). Despite the leaves' varying differences, the two kinds serve to provide mechanical protection, provide shade, condense fog and reduce water loss in the stem (Ventura-Aguilar *et al*., 2017).

Figure 5: Glochids and spines on a cladode (Schwartzman, 2012)

Cactus stems have several rows of collenchyma that are thicker than C3 and C4 plants by eight-fold (Ventura-Aguilar *et al*., 2017). These rows are succeeded by chlorenchyma layers which are responsible for photosynthesis (Prat & Franck, 2018) and the storage of phenolic compounds (Ventura-Aguilar *et al*., 2017). The parenchyma is found in the centre of the stem and is responsible for storing water (Prat & Franck, 2018). In addition to the parenchyma, scattered mucilage channels can be found. Gibson and Nobel (1986) as cited by Prat and Franck (2018) have defined mucilage as "very slippery, complex and formed by indigestible carbohydrates". Furthermore, it makes up approximately 13% of the stems' dry weight and has the ability to retain 30% of the water found in the parenchyma (Ventura-Aguilar *et al*., 2017).

2.3.2. Chemical, physicochemical and nutritional properties

Cactus stems are highly appreciated for their various chemical compounds which have anti-inflammatory, antioxidant, anti-carcinogenic as well as anti-mutagenic properties (Stintzing & Carle*,* 2005). The composition of the stems differs from cultivar to cultivar as a result of edaphic influences such as the season, the site of cultivation and the plant's age (Stintzing & Carle*,* 2005). Cactus stems have further been regarded as a potential to being a source of food as a result of their phytochemicals, minerals, total dietary fibre (0.023-0.027 grams per gram fresh weight (g/g FW)), β-carotene (0.113-0.535 micrograms per gram fresh weight (µg/g

FW)), 18 different amino acids as well as Vitamin A (0.0183 – 0.22 milligrams per gram fresh weight per gram (mg/g FW)) (Bensadón *et al*., 2010; Sáenz *et al*., 2004; Ayadi *et al*., 2009; Shedbalkar *et al*., 2010; Pérez-Méndez *et al*., 2015). According to Mohamed-Yasseen *et al*., (1996), Mizrahi *et al*., (1997), Batista *et al*., (2003), Retamal *et al*., (1987), Rodríguez-Felix and Cantell (1988) as well as Pimienta-Barrios (1993) as cited by Stintzing and Carle (2005) the chemical composition of cactus stems is as follows (Table 1):

Table 1: The chemical composition of cladodes from the Opuntia species whose spines have been removed

	Fresh weight basis (g/100g)	**Dry matter basis (g/100g)**
Water	88 – 95	-
Carbohydrates	3 – 7	64 – 71
Ash	1 – 2	19 – 23
Fibres	1 – 2	18
Protein	0.5 – 1	4 – 10
Lipids	0.2	1 – 4

According to Stintzing and Carle (2005), the main minerals found in cactus stems are potassium (116 mg/100g FW), calcium (93 mg/100g FW) and sodium (2 mg/100g FW) respectively. Moreover, according to the literature, the mineral makeup of cactus stems alters through the various stages of development (Ventura-Aguilar *et al.,* 2017). Ventura-Aguilar *et al.* (2017) reported a continuous increase in levels of iron and calcium in stems ranging between 40 and 135 days old while manganese, phosphorus and zinc did not exhibit alterations as a result of age (Hernández-Urbiola *et al.,* 2011). The changes in calcium levels over time may be attributed to the formation of druses which are bountiful in the progressive stages of the stems' development (Ventura-Aguilar *et al*., 2017).

A distinctive feature of cactus stems is their acidic taste. Because of their CAM-oriented nature, it becomes critical to harvest the stems at the right time of the day as the acidity fluctuates as the day progresses. Corrales-Garcia *et al.* (2004) conducted a study on ten different cultivars of cacti and reported that those that were harvested in the early morning (06:00) displayed 50% acidity levels higher than those which were harvested in the afternoon. Another study showed that acidity is also influenced by the season, whereby cacti which were harvested in the springtime exhibited a lower acid content (30 micro equivalents per gram fresh weight (µeq g^{-1} FW)) as opposed to those that were harvested in the wintertime (90 µeq g^{-1} FW) (Stan *et al.,* 1974). As a result of the aforementioned, Ventura-Aguilar *et al.* (2017), have advised that cladodes must be harvested a few hours (2-3 hours) after dawn as the acidity will be at a lower range which will make them more suitable for consumption.

In consonance with McConn and Nakata (2004), Teles *et al.,* (1994a), Jianqin *et al.,* (2002) and Teles *et al.,* (1994b), the composition of organic acids of cactus stems which were harvested in the morning (06:00) and the evening (18:00) is as follows (Table 2):

Table 2: The organic acid composition of cladodes from the Opuntia species harvested at different times of the day

Fresh weight (g/100g)		
	6 a.m.	**6 p.m.**
Oxalic acid	35 (dry weight mg/100g)	35 (dry weight mg/100g)
Malic acid	985	95
Citric acid	178	31
Malonic acid	36	Traces
Succinic acid	Traces	Traces
Tartaric acid	Traces	Traces
Phorbic acid	Not quantified	Not quantified
Piscidic acid	Not quantified	Not quantified
Eucomic acid	Not quantified	Not quantified

The content of the various organic acids found in cactus stems changes throughout the various developmental stages of the plants. For one, Teles *et al.* (1984; 1994a) have reported that older cladodes contain lower levels of citric acid (31 mg/100g FW) as opposed to the younger ones (178 mg/100g FW); malonic acid was depleted entirely in older cladodes. On the other side of the spectrum, acids such as piscidic acid increase fourfold as the plant ages.

Located in cactus plant vacuoles, are secondary constituents called polyphenols. They serve as antioxidants that modulate various receptor cells' and enzymes' activities and counteract oxidative stress (Manach *et al.,* 2004; Shahidi, 2004). According to Guevara-Figueroa *et al.* (2010), Avila-Nava *et al.* (2014), Souza *et al.* (2014), Astello-García *et al.* (2015), Ben-Saad *et al.* (2017) as well as Msaddak *et al.* (2017), the prime phenolic compounds found in cactus stems are as follows (Table 3):

Table 3: The predominant phenolic compounds found in cactus stems

Cultivar/origin	**Age/length (cm)**	**Phenolic compounds**	**Method employed**
Opuntia spp. cv. Tapon-II, México	NA	Gallic, coumaric (140.8μg g^{-1}), 3,4-dihydroxybenzoic, 4-hydroxybenzoic, ferulic (347.7mg g^{-1}),and salicylic acid	HPLC-MS/MS
O. ficus-indica cv. Milpa Alta, México	17-20 cm	Quercetin, kaempferol, isorhamnetin	HPLC-MS
O. ficus-indica. Brazil	NA	Flavonoids	Thin layer chromatography (TLC) on silica gel plates
Opuntia ficus-indica of cultivars Copena V1, Tojo vigor and Atlixco, México	Young cladodes of 15-20 cm	Eucomic acid; chlorogenic acid; quercetin 3-*O*-rhamnosyl(1-2)-[rhamnosyl-(1-6)]-glucoside; isorhamnetin *O*-glycosides;	HPLC-MS/MS

		isorhamnetin 3-*O*-(pentosylglucoside)-7-*O*-glucoside; 3-*O*-rutinoside; isorhamnetin3-*O*-pentose	
Opuntia ficus-indica. Gafsa, Tunisia	2 -3 weeks	Phenolic acids as gallic acid, catechin, caffeic acid, epicatechin, vanillic acid, and coumarin. Flavonoids as rutin, isorhamnetin, quercetin and kampferol	HPL
Spineless cladoes from *Opuntia ficus-indica* f. inermis, Gabes, Tunisia	NA	Quercetin, quercetin 3-*O*-glucoside, kaempferol, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-rutinoside, isorhamnetin, isorhamnetin 3-*O*-glucoside, isorhamnetin 3-*O*-neohesperidoside, 3,3′,4′,5,7-pentahydroxy-flavanone, phenolics *p*-coumaric acid, zataroside-A	HPLC/MS
NA, not available.			

As with all the constituents mentioned above, the content of phenolic compounds varies depending on the area of cultivation and cultivar. Additionally, they vary as a result of developmental stages, the kind of tissues they possess, handling procedures implemented postharvest as well as the cooking methods employed before consumption (Ventura-Aguilar *et al.,* 2017). Ventura-Aguilar *et al.* (2017) have noted greater concentrations of phenolic compounds in the chlorotic parenchyma (2931 mg g^{-1} as gallic acid equivalents) in cactus stems as opposed to the parenchyma (1176 mg g^{-1} FW). Moreover, the young cactus stems contain more phenols (3945.92 µg g^{-1} FW) as opposed to the commercially sized stems (3057 µg g^{-1} FW).

González-Montero *et al.* (2009) explain that the high content of phenolic compounds in the early developmental stages of cactus stems is a result of them acting as a protective mechanism for the growing stems.

Pauly *et al.* (2013) have noted that higher plants are sheathed in a wall that contains several secondary metabolites as well as polymers which include cellulosic and non-cellulosic polysaccharides. These polysaccharides are further classified into soluble fibre (e.g. mucilage and pectin) and insoluble fibre (e.g. cellulose and lignin) (Rani & Kawatra, 1994). The former is responsible for preventing tissue desiccation by forming a colloidal system between pectin and water; the latter serves as a barricade that restricts pathogenic microorganisms' attacks on the plant (Ventura-Aguilar *et al.*, 2017). In cactus stems, fibre's principal functional properties include water retention capabilities, ion exchange, degradation of bacteria as well as the adsorption of organic compounds (Valencia & Román, 2004; Rosado-Loría, 2012). Determinants of fibre content include cultivar, area of growth, developmental stage as well as the methods employed when processing cactus stems (Ventura-Aguilar *et al.*, 2017). Ventura-Aguilar *et al.* (2017), further note reports which provide evidence of the aforementioned. For example, the stems of the *Opuntia humisfusa* cultivar from Korea have been noted to have greater contents (503.3 grams per kilogram dry weight (g kg^{-1} DW)) of total dietary fibre as opposed to the Mexican *Opuntia ficus-indica* (330 – 450g kg^{-1} DW). The variations between the two cultivars are a result of the fact that the former grasps 80 – 90% of water in the spring and summertime due to relatively high levels of humidity while it desiccates in the wintertime. On the other hand, the latter is exposed to steady temperatures in the Mexican climate which is often coupled with relatively low humidity levels and slight temperature changes between the winter and summertime. As such, Jun *et al.* (2013) have reasoned that *Opuntia humisfusa*'s exposure to environmental stress may result in an increase in insoluble fibre in the stems. Cactus stems that are 12 days old have been found to have a higher content of total fibre (383g kg^{-1} Dry Weight) than those that are 30 days old (337g kg^{-1} Dry Weight). Nuñez-Lopéz *et al.* (2013), have hence advised that young stems be consumed for nutrient absorption acceleration in the gut as well as blood sugar regulation. It is a result of these nutritional benefits that cladodes have been noted to be a potential source of fibre in the food industry. One such example of a product that has received attention is cladode flour.

This kind of flour has been reported to be economical while presenting highly nutritional components such as dietary fibre, calcium as well as potassium (Sáenz *et al.*, 2002b). The total dietary fibre content found in the flour amounted to 43% of which 28.45% constituted of insoluble fibre while 14.54% was soluble fibre, with a protein content of 3.9% (Sáenz, 1997; Sáenz, 2000). Further noticeable nutritional information found in the cladode flour included contents of ash (20 – 22%), carbohydrates (52 – 53%), lipids (0.25%), protein (15 – 16%) and water (9.75%) (Stintzing & Carle, 2005). The advantages that were reported in the use of this flour include low water activity, water absorption ability and high viscosity (Du Toit, 2017).

Cactus stems are characterised by a slimy substance called mucilage. Matsuhiro *et al.* (2006) have reported that this complex polysaccharide is made up of various monomers such as a ratio of 2:1 of L-arabinose: D-xylose, D-galactose as well as an equimolar concentration of L-rhamnose:galacturonicacid. Mondragón-Jacobo & Méndez-Gallegos (2017) have reported that the release of the polysaccharide in the stems is a response to injury and the quantity that is released is dependent on the age, the point of dehydration and variety. Ventura-Aguilar *et al.* (2017) have noted that the mucilage content from *Opuntia ficus-indica* species is 63% higher than that of wild species such as *O. megacantha albicarpa* and *O. streptacantha.* Furthermore, they mention that the polysaccharide's viscosity is higher in older cactus stems

(200g) as opposed to the younger ones (60g). Storage temperatures which vary by 10°C between the day and the night also induce an increase in mucilage as it conserves the tissue integrity by slowing down the damage brought about by the temperature changes (Goldstein & Nobel, 1991). Mucilage has since been investigated for its use as a functional ingredient due to its ability to offer viscosity to products as well as its water-holding and absorption capacity (Sáenz, 2000; Sáenz *et al.,* 2004).

2.3.3. The significance of Opuntia ficus-indica as a vegetable crop

Nopalitos have been picked and consumed as a green vegetable for centuries on end in Latin American countries. They are highly regarded as a good source of fibre and for the fact that their nutritional composition is easily comparable to that of prevalent green vegetables such as spinach and lettuce (Mizrah *et al*., 1997). Nopalitos have been consumed and prepared in various ways and times over the years. For example, they are traditionally consumed either fresh, cooked or pickled (Nerd *et al.,* 2001); in the Christian liturgical calendar, they form part of the Lenten season where they are cooked notably in the Holy Week and on other days of the year, they are marinated (Mohamed-Yaseen *et al.,* 1996). Moreover, the young stems have formed part of ingredients in dishes such as those mentioned in Table 4 (Corrales-García & Flores-Valdez, 2003; Sáenz *et al.,* 1996):

Table 4: Food and sub-products from cactus pear fruits and stems

Fruit	**Cladodes**
Juice and nectars	Juices
Marmalades	Pickles and brine
Gels	Marmalades
Jellies	Jellies
Fruit and dried blades	Flours
Sweeteners	Liquors
Alcohols, wines and vinegars	Candy
Canned fruit	Sauces
Frozen fruit and pulp	Dietary fibre
Pigments	Salads (young prickly pads)
Forage pulp (peel and seeds)	Mucilage (insoluble fibre)
	Pigments
	Roasted for cattle forage

The utilisation and consumption of nopalitos are entrenched in Mexico due to its gastronomic culture because of the ease at which cactus pear plants are cultivated and the productivity is immense (Mondragón-Jacobo & Méndez-Gallegos, 2017). It is predicted that the plant will soon break into even more markets as a result of its ever-booming functional properties being discovered. Mondragón-Jacobo and Méndez-Gallegos (2017) have noted that nopalitos exemplify ancient Mexican horticultural wisdom which is premised on the fact that farmers are able to harvest and produce good quality products all year round by understanding the vegetative structures and physiology of plants. The interpolation of nopalitos as functional vegetables in new cultures and countries has presented quite a challenge, although they are highly adaptable and productive. The impedance comes as a result of the presence of thorns, the lack of organoleptic allure once cooked as well as the plethora of mucilage (Mondragón-Jacobo & Méndez-Gallegos, 2017). Mohamed-Yasseen *et al.* (1996) have reported the vegetable's availability in stores throughout the United States of America, primarily in Arizona, California and Texas. In essence, the vegetable can be found in areas that Hispanic people inhabit.

There are three main cultivars of nopalitos which have been described by Mondragón-Jacobo and Méndez-Gallegos (2017) that are cultivated on a commercial scale as vegetables in central and northern Mexico. The first one is the tropical/subtropical 'Atlixco' *O. ficus-indica* (L.) Mill which originated in the highlands of central Mexico, however, was named 'Atlixco' after the town where its cultivation commenced. Currently, an approximated 800 hectares are sown, predominantly in the neighbouring states of Mexico City. The cultivar is robust, and upright, with intensely green cladodes which are spineless and quadrilateral. The nopalitos are known to be ellipsoidal in shape, effortless to clean and thicker than those of 'Milpa Alta' cultivar.

The cultivar has sizable fruits which have orange skin, yellow pulp and weigh approximately 180g or more. The eating quality is satisfactory, however, is of lesser standard compared to other fruit cultivars. The older cladodes are normally utilised as forage. Méndez-Gallegos and Mondragón-Jacobo (2011) as cited by Mondragón-Jacobo and Méndez-Gallegos (2017) conclusively noted that this cultivar could reach 400 tonnes ha^{-1} year^{-1} of fresh matter under intensive cultivation which deems it a highly productive cultivar. Furthermore, it can still produce sizeable fresh matter during field cultivation in highlands when grown under plastic tunnels.

The second cultivar that Mondragón-Jacobo and Méndez-Gallegos (2017) have noted is 'Copena V1'. Its genetic makeup was characterised by the late Dr. Facundo Barrientos at Chapingo Mexico. Appertaining to its characteristics, this cultivar is vigorous, spineless, has a rapid erect growth and elliptical-shaped cactus stems. Though harvested mainly for its nopalitos, this cultivar's fruits have become a point of interest due to their attractiveness. The fruits are oval-shaped, purple in colour and are usually harvested in August in Central Mexico (Herrera *et al.,* 2006).

The last cultivar that was noted is the indigenous 'Milpa Alta' *Opuntia ficus-indica* (L.) Mill. This cultivar originates from the state of Hidalgo, Mexico, in the region of the Otomi ethnic group (Reyes *et al*., 2004). It received its commercial name of 'Milpa Alta' as a result of the region wherein extensive production of nopalitos commenced. The cultivar has been widely accepted as a result of its tender stems as such, it has been deemed as the most significant for vegetable production (Herrera *et al.,* 2006). Its oblong stems are characterised by a bright green colour; they are thin and easy to peel. The plant is vigorous, vertical and highly productive yet highly sensitive to frost. Its fruits are ovoid, medium to large sized, yellow-orange in colour and of overall acceptable quality. This cultivar is estimated to cover 7 500 hectares in Central Mexico mainly in the states of Morelos, Mexico, Hidalgo and the Federal District (Herrera *et al.,* 2006). Gallegos and Mondragón-Jacobo (2011) further noted that the cultivar is well adapted to semi-arid subtropical climates and subtropical lowlands and is in essence, consubstantial to the spineless cultivars of yellow-orange fruit present in various parts of the world such as Italy, Morocco, South Africa and Tunisia.

2.3.4. Processing and postharvest technology

The official statistics report of nopalitos farming in Milpa Alta and Tlalnepantla afforded the crop its formal status in the 1980s (Mondragón-Jacobo and Méndez-Gallegos, 2017). Since then, a combination of factors such as demographics, urbanisation, continuous research and the discovery of the stems' multiple properties, has resulted in the spread of the plant in various parts of the world. It has been reported that by the year 2010, the area of cultivation had grown from 5 269 to 12 201 hectares (Mondragón-Jacobo and Méndez-Gallegos, 2017).

When compared with other vegetable crops, the area in which nopalitos are planted is currently ranked 12th, which is an attestation of its significance (SIAP, 2015). Despite their bourgeoning market in Mexico, nopalitos' consumption has not reached similar levels in other countries. Mondragón-Jacobo and Méndez-Gallegos (2017) have noted several reasons that may explain the aforementioned. Firstly, a lack of variety in methods of preparing nopalitos and the beneficial properties associated with regular consumption. Secondly, the cooked vegetables lack allure. Thirdly, the presence of spines and glochids on the stems has discouraged consumers from buying and consuming nopalitos. Lastly, the release of mucilage after cooking has also deterred the vegetable's overall acceptability by consumers.

A part of changing perceptions of consumers towards the use and consumption of nopalitos involves educating them about the basic principles of methods applied in order to maintain the vegetable's integrity and quality post-harvest or post-purchase. Nopalitos are governed by the CAM pathway as such, one of their striking characteristics is their acidity which fluctuates (0.1-0.5% of titratable acidity), as the day progresses (Mondragón-Jacobo and Méndez-Gallegos, 2017). Corrales-García (2010) has reported that regardless of the harvest time, the acidity of nopalitos decreases during the day and increases at night. The above is further cemented by Flores-Hernández *et al.*, (2004) who have observed that the decrease in acidity during the day with reference to light exposure is a common reaction of CAM plants. Cantwell *et al*. (1992) and Domínguez-López (1995) have observed that nopalitos must be harvested in accordance to their specific required acidity which ranges between 0.94% in the morning and 0.47% in the afternoon. Taking into consideration the aforementioned, a recommendation has been submitted by Pimentel-González (2013), who suggests that nopalitos be harvested 2 hours after sunrise. It is in this period that conditions are highly favourable for harvesting as the humidity is at its highest and workers are less likely to be exposed to glochids.

According to Corrales-Garcia *et al.* (2004), the acidity of nopalitos not only appears to be dependent on the time of harvest but also the subspecies variant (0.28% - 0.76%) as well as postharvest conditions practised. Aguilar-Sánchez *et al*. (2007) conducted a study on 21 nopalito cultivars employed as vegetables which revealed acidity levels of various cultivars as follows: 'Jalpa' and 'Morado Italiano' contained approximately 0.43%; 'Milpa Alta' contained 0.68% and 'Oreja de Elefante' which is a forage cultivar contained 0.69% acidity.

Rodríguez-Felix and Cantwell (1988) reported that although the acid content increased with the age of the nopalitos prior to being harvested, it tends to even out when the nopalitos are stored at high temperatures. Contrary to the aforementioned, Pimienta-Barrios (1993) has reported that cool storage temperatures can maintain the acid content or even amplify it.

The acid content is not the only constituent of the stems that is modified during storage. The cooling effect caused an increase in the content of protein while the pH and sugar content were reduced. Another study revealed that storing cladodes at 20°C for seven days presents a 10% weight loss after the sixth day while the content of ascorbic acid drops by 20 - 40% (Cantwell, 1995; Rodríguez-Felix & Villegas-Ochoa 1997). These fluctuations modify the flavour of nopalitos, and according to Corrales-García *et al*. (2004), the time of processing and consumption are more important than the actual harvest time.

In an effort to minimise deteriorative processes such as microbial growth, rapid enzymatic browning and physiological disorders, various methods have been employed. These methods include: i) storing the stems for 30 days at 5°C in modified pressures of up to 6.9 kPa of carbon dioxide and 8.6 kPa oxygen (Guevara *et al.,* 2001); ii) dipping the stems in an ascorbic acid solution (100ppm) or chlorinated water for 15 minutes at 4°C (Stintzing & Carle, 2005); iii) blanching at 80°C for 120 seconds or 85°C for 15 seconds (Guevara *et al.,* 2001) and iv) bisulphite, ascorbic acid or citric acid treatment (Rodríguez-Felix, 2002; Guevara *et al.,* 2003).

2.3.5. Medicinal and pharmacological characterisation

Cactus stems have been traditionally used to treat and heal various ailments for centuries on end. Their use as medicine can be traced back to the ancient Mesoamerican civilisation where they were regarded as therapeutic agents (Nazareno, 2018). It has further been noted that the stems, especially those from *O. fuliginosa* and *O. streptacantha* have been used in folk medicine for the treatment of dyspnoe, fatigue, gastritis and liver injury as a result of alcohol abuse (Hitchcock *et al.,* 1997; Shapiro & Gong, 2002a; Shapiro & Gong, 2002b). Over the years, scientific investigations by Warschkow and Warschkow (1994) as well as Hegwood (1990) have revealed positive effects of the stems on acidosis, atherosclerosis and hyperglycemia.

A decrease in cholesterol and triglycerides was once noted in the plasma of hypercholesterolemic rats which were fed *O. ficus-indica* (Nazareno, 2018). This stabilisation effect can be attributed to the high fibre and pectin content which have the ability to improve the peristaltic motility in the body (Williams, 2002; Stintzing & Carle, 2005). The anti-hyperlipidemic effect of cactus stems has been investigated by various researchers. One such study which focussed on guinea pigs revealed that the decrement of blood lipids brought about by pectin was a result of the enhanced binding of bile acid (Fernández *et al.,* 1990; Fernandez *et al.,* 1992; Fernández *et al.,* 1994). In addition, the consumption of nopalitos has been shown to reduce obesity and blood glucose (Stintzing & Carle, 2005). The anti-obesity, antidiabetic, as well as hypoglycemic effects were all detected in human and rat subjects (Bwititi *et al*., 2000; Frati Munari *et al*., 2004; Yang *et al.,* 2008). Hfaiedh *et al*. (2008) reported on the shielding effect of cactus stem juice from the *Opuntia ficus-indica* species against nickel-induced toxicity. In this experiment, it was observed that the exposure to nickel (Ni) led to the generation of reactive oxygen species (ROS) which further led to lipid peroxidation, membrane integrity loss and adaptations in the cellular antioxidant system. It was indicated that oxidative stress as well as the related parameters in rats, could be inhibited by the juice from cactus stems. Additionally, the regular ingestion of cladode juice can negate the peroxidative effects of nickel (Nazareno, 2018). Ncibi *et al*. (2008) and Zourgui *et al*. (2008) as cited by Nazareno (2018) have reported corresponding protective effects of cactus juice against oxidative damage induced by various toxoids. *O. ficus-indica* flavonoid extracts have been noted as being effective in providing neuroprotective action against neuronal oxidative injuries in cultured mouse cortical cells and to act against global ischemia in gerbils (J.H. Kim *et al*., 2006). Furthermore, Brahmi *et al*. (2011) have shown the possibility of cladode extracts having hepatoprotective effects against aflatoxicosis in mice which are achieved by boosting the antioxidant defence systems. There has been a report of a cladode extract from *Opuntia streptacantha* which was revealed to have antiviral properties towards DNA and RNA viruses such as influenza type A and human immunodeficiency virus HIV−1, respectively (Stintzing & Carle, 2005). Ahmad *et al.* (1996) have ascribed the aforementioned antiviral properties to a protein with explained mechanisms of action, whose active principle is located in the outer non-cuticular tissue.

2.3.6. Further uses of cactus stems in various industries

Cactus stems have shown remarkable potential to be used in various industries as a result of their multifacetedness. Nopalitos are known to be highly perishable when marketed fresh, however, there are specific industrial processes that may be employed to ensure prolonged longevity as they are transformed into various products. The young stems are primarily processed in brine, a pickle or they are dehydrated (www.dezeewierwinkel.nl, 2015).

Sáenz *et al*. (2010) noted that dehydrated stems were not intended for consumption but rather to be transformed into powders which can find various applications in products such as flour which may be used as an excellent gluten-free alternative. Fibre is one of the main components found in cladodes and has been deemed an essential part of the everyday diet. It is due to this reason that the nopal flour has since gained momentum as a gluten-free alternative. Saenz (2000) has reported that this kind of flour can be incorporated with the likes of wheat flour when preparing baked goods. De Wit *et al.* (2015) have further noted the incorporation of nopal flour in baked goods. This implementation has interesting applications as it increases the consumption of soluble fibre, which significantly improves overall digestion.

Moreover, the cladode powder is used in capsules to regulate blood sugar, weight as well as to proliferate fibre intake (Stintzing & Carle, 2005).

Sáenz (2000) as cited by Sáenz (2018) reported that the viscosity of powder-based products that require the addition of water or juice before consumption was highly dependent on the mucilage and pectin content found in the cladodes. These two hydrocolloids are known to absorb and retain water. As such, they find application in products such as food thickening agents in dessert gels, vegetable soups and can also be used as an ingredient in breakfast cereals (Sáenz, 2003; Stintzing & Carle, 2005). Medina-Torres *et al.* (2000) have reported that a 10 g/100g cactus polysaccharide solution was comparable to a 2 g/100g xanthan solution in terms of rheological properties. They also reported that the former's viscosity could be expanded by either reducing the ionic strength or increasing the pH. Moreover, stronger tendencies to gel formation were exhibited by a 5% solution at 35°C as a result of conformational changes in the polysaccharide.

It has been reported that mucilage has the potential to be used as an edible coating to preserve fresh fruits. Del Valle *et al*. (2005) reported strawberries to have maintained their flavour and texture after applying them with a mucilage-based edible film and storing them at 5°C. Furthermore, there were no signs of deteriorative processes after nine days of storage. A challenge has, however, arisen with the application of mucilage to various products as a result of their varying respiratory rates.

A blend of mucilage solution and different concentrations of citric acid and sodium bisulphate were used to treat banana slices in an effort to prevent browning (Aquino *et al*., 2009). The writers have reported effective browning inhibition in the banana slices following treatment of a blend of 500 ppm sodium bisulphite and citric acid (1%) in the mucilage solution (35 mPas). Moreover, the banana slices appeared shinier in appearance following the treatment.

Non-food applications of the nopalitos include the cosmetics and skin industry. Sáenz *et al.* (2002a) and Sáenz (2002b) have reported that the juice from the stems may be found in conditioners, lotions, shampoos, soaps as well as sun protectors. Warschkow and Warschkow (1994) have also made claims that the juice aids in hair growth.

Cladodes have been used as additional fodder for cows, goats and sheep in many parts of the world for periods of intense drought conditions (Stintzing & Carle, 2005). This is due to the plant's adaptable variability wherein, it remains succulent while other plants and the grass become senescent or overgrazed (Russell & Felker, 1987). Moreover, because of its high water content, it serves as a replacement for drinking water (Nazareno, 2018).

Dubeux *et al.* (2018) have reported that the plant serves as forage in various ways, with the main methods being the following: i) direct browsing where livestock directly browse through the plants; ii) cut-and-carry which is the most commonly used method involves harvesting the pads and taking them to the barn, then chopping them, mixing with other feeds and placing them in troughs; iii) drying wherein which, the pads are dried either to produce a commercial feed, mix with other ingredients or to prune due to the short harvest time; and finally, iv) silage which is employed when wet agro-industrial by-products cannot be stored for a long time and must be enhanced or the duration of production is limited. As with humans, the consumption of cladodes by animals has been shown to improve overall qualities in these animals. For one, several authors have deduced that the average daily gain (ADG) of growing ruminants increases with the supplementation of low-quality forage with fresh cactus (Nazareno, 2018). For example, Gebremariam *et al*. (2006) have reported that the ADG of Ethiopian lamb rose from 23 to 53g day^{-1} once 50% of the teff straw was replaced with mature cactus cladodes.

Dubeux *et al.* (2018) have noted an increase in the use of cacti in dairy cattle feeding due to the readily available and distribution of spineless cacti in recent decades. As reported by the farmers in northern Brazil, Holstein cattle which were fed a mixed diet of 60% chopped fresh cactus stems, 20% fibrous feedstuff (hay or straw) and 20% protein-rich concentrate produced approximately 20 litres of milk per day (H. Ben Salem, personal observation as cited by Nazareno, 2018). The replacement value of cactus (0, 12, 25, 38 and 51%) for replacing cracked corn and Cynodon hay on dairy cattle raised in northern Brazil was investigated (Oliveira *et al*., 2007). The results revealed that cactus could completely take the place of cracked corn and partially replace hay (around 40%) without affecting milk production (20.3-21.8 kg day^{-1}) significantly.

The effect of consumption of cladodes on the overall meat quality of the animals was also investigated. In one study, it was observed that replacing barley with cactus as an energy supplement in the local goat kid and Barbarine lamb diet increased the vaccenic acid content without compromising the integrity of the intramuscular fatty acid composition of meat (Abidi *et al*., 2009a and b). This fatty acid contributes positively to the cardiovascular system as all conjugated fatty acids do (Nazareno, 2018). In another study, Atti *et al*. (2006) analysed the meat quality of kids that were receiving a concentrate-based cactus diet which was slightly supplemented with oat hay. Increased proportions of linoleic, linolenic and conjugated linoleic acid were observed as well as proportions of polyunsaturated fatty acids (PUFA) and higher PUFA to saturated fatty acids (SFA) ratio. The abovementioned studies confirm that diets of sheep and goats, which were supplemented with cladodes improve the quality of meat as per consumer preference: more PUFA and less SFA (Nazareno, 2018).

One of the worldwide markets in which cactus stems find great demand is that of cochineal dye production. The pigment which is mostly extracted from *Dactylopius coccus*, is often used in food colouring, cosmetics, drugs, fabrics and several other products (Cañamares *et al*., 2006; Chávez Moreno *et a*l., 2009). Cactus stems serve as hosts for the breeding of the insects. In Mexico, it has been reported that several *Opuntia* species serve as hosts for cochineal breeding, with the most important cultivars for commercial production being the spineless ‘Atlixco’, ‘Chicomostoc’ and ‘Milpa Alta’ (Nazareno, 2018). Currently, Peru, the Canary Islands and Chile are the primary producers of the dye followed by Mexico, Bolivia, and Argentina (Méndez *et al.,* 2004).

2.4. Consumer analysis

For any food product that is to be considered for human consumption and commercialisation, an in-depth consumer analysis is required. Consumer analysis is the process in which information about consumers is investigated and compiled from market research which involves the needs of the consumer, the target market as well as the relevant demographics (MBA Skool, 2018). This information also assists in building up a database so that this information can be used in future in the market segmentation (MBA Skool, 2018). The steps that are involved in implementing a successful consumer analysis involve a thorough overview of the industry; the identification and description of demographics of the customers; a projection of future changes; the determination and description of consumer buying behaviour; competitive analysis and the use of information about industry, customer and competitors in order to identify gaps in the market (MBA Skool, 2018).

One manner in which consumer and product information can be attained is through sensory research. This discipline incorporates scientific principles which stem from food science, physiology, psychology and statistics (Piggott *et al.,* 1998). The purpose of the discipline is to obtain responses to food properties as perceived through the five senses, sight, smell, taste, touch and hearing (Piggott *et al.,* 1998). The various sensory techniques that are employed in every investigation must be accurate, valid and precise (Piggott, 1995) and must further still be related to consumer preferences and acceptance (Piggott *et al.,* 1998). Consumers play a critical role in the overall success of a food product on and off the food shelves. It is as such, that one of the main goals of sensory analysis is to understand the significance of sensory characteristics and the role that they play in the overall consumer acceptance (Piggott *et al.,* 1998). Various methods are used to investigate the sensorial characteristics of food products which are largely categorised into two groups, namely descriptive methods and discriminant methods (Piggott *et al.,* 1998). The difference between the two groups lies in that the discriminative methods indicate whether the products that are being tested can be perceived differently and distinctively, while descriptive methods employ chemical analyses in order to measure and determine the composition of the products (Piggott *et al.,* 1998). Regardless of the methods employed, sensory analysis plays a critical role in the overall acceptability, consumption and success of a food product, just like the cactus pear plant crop.

2.5. Conclusion

Young cactus stems are indigenous to Latin American countries and display anatomical, chemical, morphological and physiological adaptations which have allowed them to endure and thrive under harsh climatic conditions. In recent years, the stems have gained eminence the world over as they present multifaceted properties which have the potential to be utilised in various industries. By virtue of their high respiration rate, new methods of postharvest technology need to be investigated and employed to ensure prolonged shelf life. The plant's ability to thrive under harsh conditions which are characterised by poor soil quality, long periods of drought and extremely high temperatures makes it a potential plant that would thrive commercially in South Africa. The increasing scarcity in terms of agricultural resources such as water, has created a need to explore alternative sources of food; specifically crops that would be efficient by not adding problems to the already strained resources. The versatility of the crop warrants its application for mass production in the country for humans and animals. This will not only contribute towards the already growing momentum of agro-businesses in the country but also assist in mitigating the negative impacts of global warming.

Chapter 3

3. The morphological, physiological and physico-chemical evaluation of nopalitos from twenty South African cactus pear cultivars

Abstract

The cactus pear plant (Opuntia ficus-indica) belongs to the Cactaceae family and is regarded as a succulent plant. Cactus pear fruits are relatively well known and in certain parts of the world, such as Mexico, and are an integral part of the human diet. The fruit and modified stems (cladodes) are a vital livestock feed. The plant's ability to thrive in the harshest of environmental conditions makes it of great interest to food scientists who are interested in sustainable food sources. The objective of this study was to determine the morphological and physico-chemical properties of the young edible stems (nopalitos) from twenty South African cultivars to determine their influence on the eating quality for human use. The morphological and physico-chemical analyses were conducted over two years, i.e., 2018 and 2019. Overall, the 2018 year produced better quality nopalitos in terms of eating qualities compared to 2019. The overall cultivars of interest were Meyers, Malta, Nudosa, Fusicaulis, Fresno and Morado. The reported work has shown that nopalitos could be acceptable to consumers as a vegetable source.

3.1. Introduction

The cactus pear plant (*Opuntia ficus-indica)* produces edible stems (cladodes) and fruits with high nutritional value in terms of minerals, protein, dietary fiber and phytochemicals. With the increase in the global shift towards the use of natural plant materials in various industries including food, cosmetics as well as pharmaceuticals, the cactus pear plant has received interest from the research community (Stintzing *et al*., 2000; Sáenz, 2002; El gharras *et al.*, 2008). In addition to its multifaceted properties, the plant is all the more appealing in the South African landscape because of its high ecological adaptivity. The plant's high ecological adaptivity can be attributed to its evolution over the years, which has rendered its unique anatomical, morphological and physiological characteristics (Ventura-Aguilar *et al.,* 2017). The cactus pear plant is known to grow in different parts of the world where it has various uses. For example, in Mexico, the plant has been regarded as an imperative component of agriculture and part of the general dietary regime since the 16th century (Hernández-Urbiola *et al*., 2011). Peña-Valdivia *et al.* (2008) have reported that research on the biochemistry, genetics, morphology, physiology as well as the taxonomy of *Opuntia* species of cacti is critical in preserving the genus' genetic resources.

This will, in turn, assist in expanding the knowledge and information pertaining to the diversity of the species, the effects of the changes in the environment as well as the modifications which are a result of domestication (Peña-Valdivia *et al.,* 2008). The characterisation of cladode morphology of the *Opuntia* species and its significance thereof has been strongly emphasised by several researchers (Valdéz-Cepeda and Blanco-Macías, 2002; Reyes-Agüero *et al*., 2005a, b). The morphology of nopalitos has been effective in providing information about the *Opuntia'*s reaction to cultivation as well as its potential uses (Peña-Valdivia *et al.,* 2008); the clarification, support as well as the improvement of the taxonomy of the species (Pimienta-Barrios, 1990; Sudzuki-Hillis, 1995); the quantification of the genetic variability of *Opuntia* (Peña-Valdivia *et al*., 2008)*;* and the characterisation of intra- and inter-specific hybridisation processes in *Opuntia* (Gibson and Nobel 1986; Pimienta-Barrios and Muñoz-Urias 1995; Mondragón-Jacobo and Pérez-González 2001; Mondragón-Jacobo and Pérez-González 2002). It should be noted that little is known about the young stems' properties. As a result, this comprehensive study was conducted to investigate the young stems which are typically used for animal feed in South Africa as opposed to a functional vegetable as seen in Latin American countries. The aim of this study was, therefore, to investigate the morphological, physiological and physico-chemical properties of young stems of twenty South African cultivars of cactus pear plants.

3.2. Materials and methods

3.2.1. Collection of nopalitos

Six samples of each of the 20 cultivars were harvested at the University of the Free State's West campus in Bloemfontein which is also known as its research cactus pear orchard. The cladodes were harvested in 2018 and 2019 with the size of the nopalitos ranging between 12-20 centimetres. The harvest was done during the midmornings (09:00-11:00) as this is the time in which the acidity levels that affect the taste of the nopalitos are the lowest (Rodriguez–Felix & Cantwell., 1988). Each cladode was cut from the mother cladode in order to avoid tissue injuries using a sharp blade. The cladodes were then labelled, packaged and transported to the lab and stored at four degrees Celsius.

3.2.2. Sample preparation

Cladodes were allowed to warm to room temperature for 30 minutes, after which the thorns and the outer edges of the nopalitos were removed with a sharp blade.

3.2.3. Morphological and physiological analysis

Upon sample preparation, various analysis was conducted including:

3.2.3.1. Determination of weight (g)

After the removal of the thorns and outer edges, each nopalito was weighed on a Radwag Ps 750/c/2 scale and the mass was recorded in grams (g).

3.2.3.2. Determination of size

The diameter, width and length of each nopalito were measured using a measuring tape and recorded in centimetres (cm). In order to measure the length of each nopalito, the cladodes were measured from top to bottom. The widest part of the nopalito which runs from the left to the right of the stem was measured in order to determine the width, while the diameter was obtained by turning the nopalito on its side.

3.2.3.3. Determination of extracted mucilage yield and waste

The nopalitos were cut into small pieces and pulsed through a food processor in order to form a pulp. The pulp that was obtained served as the pulp obtained before the centrifugation process. In order to obtain maximum mucilage from the macerated nopalitos, they were cooked in the microwave oven for three minutes. Once cooked, the weight of the macerated nopalitos was noted down in grams (pulp before centrifugation). The pulp was then transferred to various centrifuge tubes and centrifuged for 15 minutes at 8000 rpm speed according to a patented method by Du Toit and De Wit (2011). Once centrifugation was completed, the liquid (extracted mucilage) and sediment (pulp regarded as waste) were weighed off separately and recorded in grams. The resulting %Yield of mucilage and %Waste was calculated from the original weight of the nopalitos and not from the pulp before extraction. The calculation is as follows:

$$\%mucilage\ yield = \frac{supernatant\ liquid\ (g)}{original\ nopalito\ weight\ (g)}\ x\ 100$$

$$\%waste = \frac{precipitated\ pulp\ (g)}{original\ nopalito\ weight\ (g)}\ x\ 100$$

3.2.4. Physical analysis

3.2.4.1. Determination of colour

Six readings per nopalito were taken using a Konica Minolta Chroma CR-400 meter in order to measure the three CIELAB coordinates (L*, a*, b*) of the different nopalitos. The colorimeter was placed on the top round of the nopalito, in the middle and either the left or right side from the middle. The nopalitos were free of thorns at that stage and the colour measurements could thus not be tainted. The L* coordinates measured the lightness of the colour of the cladodes; a value of 0 signified a dark colour in the cladodes while a value of 100 signified a light colour (Konica Minolta, 2007). The a* coordinates measured the redness or greenness found in the cladodes; positive a* values signified a red colour while negative a* values signified a green colour (Konica Minolta, 2007). The b* coordinates measured the yellowness or blueness found in the cladodes positive b* values indicated a yellow while negative b* values signified a blue colour (Konica Minolta, 2007). The C* coordinates measured the relative saturation of colours whereby a value of 0 signified complete unsaturation while a value of 100 signified a pure/bright colour in the cladodes (Konica Minolta, 2007). The h° coordinates measured the position of colour on a colour wheel in degrees. 0° signified red; 90° signified yellow; 180° signified green and 270° signified blue. It must be noted that the C* & h° values were calculated using the below calculations (Konica Minolta, 2019):

$$Metric\ Chroma\ (C^{*}) = \sqrt{(a^{*})^{2} + (b^{*})^{2}}$$

$$Metric\ hue\ angle\ (h^{\circ}) = \tan^{-1}(\frac{b^{*}}{a^{*}})\ \text{(degree)}$$

3.2.4.2. Determination of compressibility and firmness

The Stanhope-seta penetrometer set at 5-second intervals was used in order to measure the compressibility and the firmness of the nopalitos. With regards to the compressibility measurements, a cylinder probe which had a flat surface was used. During this test, the flat surface was placed right in the centre of each nopalito and pushed down. The compressibility of the nopalitos was measured in millimetres (mm) whereby a high value indicated nopalitos which had a much more soft tissue while a low value indicated nopalitos which had hard tissue. In terms of firmness, three measurements were taken per nopalito using an 18500-0 Seta penetration needle. Measurements which were taken included one on the right side from the centre; one in the centre of the nopalito and another one on the left side of the centre. High values indicated thin and flexible nopalitos while low values indicated firm nopalitos.

3.2.4.3. Determination of mucilage viscosity

The viscosity of the resulting mucilage extracted from the 20 cultivars that were obtained was analysed in two ways. In the first method, a calibrated Brookfield DV3T viscometer was used whereby the sample container was filled with 7 ml of mucilage before being attached to the viscometer. A spindle with the number sc4-21 was attached to the viscometer and immersed into the mucilage. Using 7 ml of mucilage, the system ran seven steps at different speeds for a period of 1 minute and 45 seconds. The viscosity values that were taken correlated to the highest torque values, which were required to be at least 10% (Brookfield Ametek, 2019). The line-spread method, which was the second method employed in the investigation of viscosity, was conducted using 5 ml of mucilage. The mucilage was placed into a cylinder that was placed on the line spread surface in the middle. The cylinder was then lifted up in order for the mucilage to flow freely over the surface that is marked with numbers ranging from 1 to 13cm with 0.5cm intervals. These intervals were used to determine the distance it takes for the mucilage to flow across a flat surface. The sum of the centimetres whereby the mucilage stopped running was then used to calculate the resulting viscosity. Higher values of the line-spread test indicated lower mucilage viscosity while lower values indicated highly viscous mucilage (Kim *et al.*, 2014).

3.2.5. Physico-chemical and chemical analysis

3.2.5.1. Determination of pH

Following the physical measurements of the nopalito cultivars, they were macerated in a food processor and placed in containers for further analysis. pH measurements were done using a calibrated Eutech pH 2700 pH/mV/˚C/˚F instrument at 22°C.

3.2.5.2. Determination of moisture and solids

Petri dishes were weighed without and with nopalito plant material, placed in an Ecotherm oven set at 102 °C and then left for 24 hours to dry. Moisture (%) and solids (%) were calculated as follows:

$$Moisture\ content\ (\%) = (original\ sample\ (g)\ -dry\ sample\ (g))/\ (original\ sample)\ \mathrm{x}\ 100$$

$$Solids\ content\ (\%) = 100 - moisture\ content\ (\%)$$

3.2.5.3. Determination of sugar content (Total Soluble Solids, TSS)

The sugar content found in the mucilage was determined using a handheld Atago N-1α refractometer. One millilitre of mucilage was poured onto the surface of the daylight plate, and the top was closed and lifted into the light to see the exact measurement of the specific mucilage. The resulting values were measured in degrees Brix (°Bx).

3.2.5.4. Determination of titratable acidity (TA)

Using a pipette, 5 ml of the mucilage was drawn up and transferred to a reaction vial. Thirty-five millilitres of distilled water was added to the vial upon which 3 ml of the phenolphthalein indicator was added to the mixture. Using a clean pipette, 10 ml of sodium hydroxide was titrated at a rate of 0.5 ml at a time, and the vial was gently shaken. The point at which a faint pink colour appeared in the solution was the endpoint of the reaction and the amount of added sodium hydroxide was recorded. The final titratable acidity was expressed as %TA expressed as citric acid, which was calculated as follows:

$$\%\ Titratable\ acidity\ expressed\ as\ citric\ acidity:$$

$$= \frac{titre\ (ml) * 192.12\ (Molecular\ weight\ of\ citric\ acid)}{3\ (ml\ of\ phenolpthalein\ indicator)\ x\ 1000}$$

3.3. Statistical analysis

The effect of cultivar, year of harvest and the interaction between cultivar and year of harvest on the various morphological, physiological and physico-chemical parameters over the two years were determined by analysis of variance (ANOVA) (NCSS 11 Statistical Software 2016). The means were compared with the Tukey-Kramer multiple comparison test at $\alpha = 0.05$ (NCSS 11 Statistical Software 2016).

3.4. Results and discussion

Pursuant to literature, the ideal commercial raw nopalito must be thin, and turgid, look fresh in appearance, and have a bright green colour (Ruiz-Pérez-Cacho *et al.*, 2005). Good quality nopalitos are low in acidity and mucilage content as these traits would have a negative influence on the eating quality (Razo & Sanchez, 2002). Bearing in mind the aforementioned characteristics and the purpose of this chapter, which seeks to investigate the morphological, physiological and physico-chemical properties of the nopalitos in relation to their overall eating acceptability, the various attributes will be highlighted below.

Tables 5 - 9 show the effects of cultivar and the year of harvest on the various attributes of the nopalitos. Because the climate of the area of cultivation is one of the key factors that causes variation in the composition of nopalitos, it was deemed imperative to further show the effect of the two different years on the various attributes of the nopalito cultivars in detail. The two years that were investigated in this regard were 2018 and 2019, thus the data in Tables 10 to 12 show the means of two years of data collection. Furthermore, the interaction between the cultivar and the year of harvest on the various properties can be seen in Tables 13 – 17. In general, various significant differences were observed from the results obtained.

Table 5 depicts the ANOVA for the influence of the cultivar, the year of harvest as well as the cultivar and the year of harvest interaction on the various attributes.

Table 5: Analysis of variance (ANOVA) for the influence of cultivar, year of harvest, as well as the cultivar and year of harvest interaction on the various nopalito attributes

Attribute	**Cultivar**	**Year**	**Cultivar X Year**
Weight (g)	p < 0.001	p = 0.887	p = 0.180
Length (cm)	p < 0.001	p = 0.981	p = 0.292
Width (cm)	p < 0.001	p = 0.141	p = 0.071
Diameter (cm)	p < 0.001	p = 0.915	p = 0.100
pH	p = 0.389	p < 0.001	p = 0.072
Compressibility(mm)	p = 0.468	p < 0.001	p = 0.011
Firmness (mm)	p < 0.001	p < 0.001	p = 0.420
Moisture content (%)	p = 0.966	p = 0.730	p = 0.999
% Solids	p = 0.966	p = 0.730	p = 0.999
Pulp (before centrifuge) (g)	p < 0.001	p < 0.001	p = 0.046
Extracted mucilage (g)	p < 0.001	p = 0.004	p < 0.001
Waste (g)	p < 0.001	p < 0.001	p = 0.036
% Mucilage yield	p < 0.001	p < 0.001	p < 0.001
% Waste	p = 0.069	p < 0.001	p = 0.064
Viscosity (cP)	p = 0.061	p < 0.001	p < 0.001
Titratable Acidity expressed as % citric acid	p < 0.001	p < 0.001	p < 0.001
Line-spread (cm)	p < 0.001	p = 0.945	p < 0.001
Sugar (TSS °Brix)	p < 0.001	p = 0.055	p < 0.001
L*	p < 0.001	p < 0.001	p < 0.001
a*	p < 0.001	p < 0.001	p < 0.001
b*	p < 0.001	p < 0.001	p < 0.001
Chroma (Saturation Index)	p < 0.001	p < 0.001	p < 0.001
Hue Angle	p < 0.001	p = 0.003	p < 0.001

Means with different superscripts in the same column differ significantly

In Table 5, significant (p < 0.001) differences were observed in the influence of cultivar on 17 attributes out of a total of 23. The 17 attributes are the weight (g), length (cm), width (cm), diameter (cm), pulp (before centrifuge) (g), extracted mucilage (g), waste (g), % mucilage yield, firmness (mm), titratable acidity expressed as % citric acid, line-spread (cm) method of determining viscosity, sugar (TSS °Brix) content as well as the colour coordinates of the various nopalito cultivars (L*, a*, b*, Chroma (Saturation Index) and hue angle).

The six attributes which were not significantly affected by the various cultivars are the pH, compressibility (mm), moisture content (%), % solids, % waste and viscosity investigated using a viscometer (cP).

The influence of the year of harvest on the various cultivar attributes also showed significant ($p < 0.001$), ($p = 0.003$) & ($p = 0.004$) effects on 15 attributes out of a total of 23. These attributes are the pH, compressibility (mm), firmness (mm), pulp obtained before centrifugation (g), extracted mucilage yield (g), waste (g), % mucilage yield, %waste, viscosity using a viscometer (cP), % titratable acidity expressed as citric acid as well as the colour coordinates (L*, a*, b*, Chroma Saturation Index and hue angle) of the various nopalito cultivars. The effect of the year of harvest did not show any significant differences in the weight (g), length (cm), width (cm), diameter (cm), moisture content (%), % solids, line-spread method of obtaining viscosity (cm) and sugar content (TSS °Brix) of the various cultivars over the two years.

With regards to the influence of the cultivar and year of harvest interaction on the various attributes, significant ($p < 0.001$), ($p = 0.036$) & ($p = 0.011$) differences were observed in 14 attributes out of a total of twenty-three. These attributes are the compressibility (mm), pulp before centrifugation (g), extracted mucilage yield (g), waste (g), % mucilage yield, viscosity obtained using both a viscometer (cP) and the line-spread method (cm), %titratable acidity expressed as citric acid, sugar content (TSS °Brix) as well as the colour coordinates of the various nopalito cultivars (L*, a*, b*, Chroma (Saturation Index) and hue angle). The effect of the cultivar and year of harvest interaction did not show any significant differences in the weight (g), length (cm), width (cm), diameter (cm), pH, firmness (mm), moisture content (%), % solids as well as the % waste of the various nopalito cultivars. The extent to which each parameter is influenced by the different cultivars will be discussed in the paragraphs to follow.

Table 6 shows the morphological characteristics and the yield of pulp and mucilage from the 20 nopalito cultivars.

Table 6: Analysis of variance (ANOVA) for the influence of cultivar morphological and physiological attributes of nopalitos harvested over two years

Cultivar	Weight (g)	Length (cm)	Width (cm)	Diameter (cm)	Pulp (before centrifuge) (g)	Extracted mucilage (g)	Waste (g)	% Mucilage yield	% Waste
Gymno Carpo	68.98^{ab} ± 22.70	16.03^{a} ± 2.15	9.00^{abcde} ± 1.57	0.43^{a} ± 0.14	46.11^{ab}±22.64	11.26^{ab}±6.79	30.00^{abc}±16.97	15.94^{abc}±6.36	44.54 ±19.65
R1251	64.61^{ab}± 16.11	16.92^{ab}± 2.33	8.28^{abc}± 1.01	0.44^{a}± 0.11	32.79^{a}±14.74	12.75^{ab}±9.48	23.31^{a}±7.93	18.20^{abc}±11.73	36.64±11.49
Meyers	77.16^{ab}± 21.29	17.52^{ab}± 1.86	9.58^{bcdef}± 1.68	0.48^{a}± 0.12	48.49^{ab}±24.70	22.21^{ab}±13.33	26.20^{ab}±11.08	23.93^{abc}±12.05	34.47±11.14
Skinners Court	70.24^{ab}± 25.97	17.34^{ab}± 2.15	7.33^{a}± 1.72	0.48^{a}± 0.15	40.92^{ab}±30.07	10.66^{ab}±19.50	28.03^{abc}±11.87	11.08^{a}±16.93	41.00±11.05
Algerian	84.03^{ab}± 26.55	17.68^{ab}± 2.32	9.58^{bcdef}± 1.35	0.50^{a}± 0.09	55.70^{ab}±24.36	17.94^{ab}±9.28	33.07^{abc}±9.99	20.33^{abc}±8.67	41.84±12.94
Ofer	60.07^{a}± 17.38	16.14^{ab}± 1.81	8.43^{abcd}± 1.16	0.44^{a}± 0.11	35.59^{a}±20.67	7.87^{a}±8.97	26.74^{ab}±10.98	11.31^{a}±9.48	44.12±11.65
Turpin	66.45^{ab}± 25.62	16.49^{ab}± 2.14	8.76^{abcd}± 1.82	0.48^{a}± 0.17	35.82^{a}±16.07	11.28^{ab}±8.43	24.91^{a}±14.52	15.90^{abc}±10.11	38.10±19.39
Messina	78.73^{ab}± 38.74	16.36^{ab}± 2.95	8.18^{abc}± 1.54	0.53^{a}± 0.17	47.30^{ab}±37.76	18.69^{ab}±22.49	28.25^{abc}±14.60	16.89^{abc}±14.09	37.78±11.12
Santa Rossa	109.61^{bc}± 48.66	19.63^{b}± 2.42	10.23^{cdef}± 1.96	0.42^{a}± 0.14	78.15^{abc}±60.89	37.29^{bc}±36.09	31.66^{abc}±18.11	27.24^{abcd}±20.20	28.90±10.52
Berg X Mexican	67.48^{ab}± 22.95	15.99^{a}± 2.28	8.81^{abcd}± 1.40	0.46^{a}± 0.16	39.14^{a}±18.31	14.83^{ab}±8.61	23.26^{a}±9.58	20.90^{abc}±6.31	36.20±13.00
R1260	75.67^{ab}± 41.15	17.28^{ab}± 3.89	8.65^{abcd}± 2.19	0.46^{a}± 0.18	49.77^{ab}±38.36	21.92^{ab}±19.98	26.88^{ab}±16.90	26.36^{abcd}±13.42	34.38±17.21
Fresno	155.60^{c}±40.37	19.39^{ab}±2.78	10.48^{def}±1.57	0.89^{b}±0.18	71.58^{abc}±24.30	34.23^{abc}±21.38	48.71^{bc}±33.21	26.42^{abcd}±16.68	26.10±15.94
Fusicaulis	149.53^{c}±51.41	17.45^{ab}±2.68	9.13^{abcdef}±1.74	0.96^{b}±0.16	129.00^{d}±45.94	69.63^{d}±33.21	51.03^{c}±17.72	42.27^{d}±14.74	38.07±10.38
Malta	65.72^{ab}±19.04	16.82^{ab}±2.18	8.95^{abcd}±1.07	0.47^{a}±0.08	39.35^{a}±16.24	9.49^{ab}±7.46	28.42^{abc}±9.43	13.18^{ab}±7.93	44.19±11.68
Morado	63.97^{ab}±25.77	16.23^{ab}±3.10	8.75^{abcd}±1.78	0.43^{a}±0.12	35.66^{a}±14.74	9.54^{ab}±5.35	23.75^{a}±8.41	14.54^{ab}±6.31	40.32±13.64
Nudosa	153.13^{c}±54.85	19.37^{ab}±2.48	11.26^{f}±1.41	0.84^{b}±0.27	112.86^{cd}±49.36	59.07^{cd}±44.99	50.74^{c}±17.40	33.29^{cd}±12.44	37.69±10.73
Robusta	151.03^{c}±44.75	17.10^{ab}±2.32	11.15^{ef}±0.85	0.88^{b}±0.27	85.50^{bcd}±48.78	27.69^{ab}±10.59	40.87^{abc}±24.80	29.34^{bcd}±16.07	29.51±21.56
Zastron	67.65^{ab}±17.15	16.83^{ab}±1.89	7.82^{ab}±0.89	0.52^{a}±0.19	40.57^{ab}±19.04	10.38^{ab}±6.78	29.84^{abc}±10.02	14.66^{ab}±8.92	44.11±11.03
Rossa	71.34^{ab}±22.01	16.54^{ab}±1.60	8.66^{abcd}±0.98	0.46^{a}±0.13	40.60^{ab}±23.41	13.21^{ab}±11.69	27.31^{ab}±14.86	16.61^{abc}±10.65	38.58±17.39
Sicillian Indian Fig	78.11^{ab}±22.13	16.91^{ab}±2.17	8.77^{abcd}±1.08	0.57^{a}±0.17	46.63^{ab}±18.43	15.30^{ab}±9.41	30.20^{abc}±13.09	18.44^{abc}±9.11	41.50±20.53
Average	88.96	17.20	9.09	0.56	55.58	21.76	31.65	20.84	37.90
Sign level	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.069$

Means with different superscripts in the same column differ significantly

3.4.1. The effect of cultivar on the morphological and physiological attributes of nopalitos harvested over two years

3.4.1.1. Weight

Weight measures how heavy an object is. Significant ($p < 0.001$) differences in this attribute were observed in the effect of cultivar on the various properties of the nopalitos (Table 5 & 6). The weight of the various nopalito cultivars ranged between 60.07 g (Ofer) and 155.60 g (Fresno) with an average weight of 88.96 g. The majority of the nopalito cultivars did not differ significantly with regard to their weight, with many of them weighing between 60 and 85 g. Santa Rossa (109.61 g), Fusicaulis (149.53 g), Robusta (151.03 g) and Nudosa (153.13 g) also weighed significantly heavier than the rest of the nopalitos as with Fresno (155.60 g) which weighed the heaviest. Ofer weighed significantly ($p < 0.001$) less than Fresno, Fusicaulis, Nudosa and Robusta.

3.4.1.2. Size

The dimensions of cladodes have been deemed as a possible ploidy indicator (Muñoz-Urias *et al*., 1995). This was due to an observation of the commonality of higher fruits, in which cladodes and stomata were said to exist in polyploid (6n, 8n) rather than diploid in *Opuntia* plants (Muñoz-Urias *et al*., 1995). It has further been reported that a large gradient exists among the various species of cladodes in the wild, which implies that the size of nopalitos is dependent on the species being observed (Bravo, 1978). Length measures how long an object is. Flores-Valdez (1995) has noted that nopalitos that are to be utilised as vegetables must be harvested between the lengths of 15 cm – 20 cm. It must be noted that the length of the harvested nopalitos was specific (12 cm – 20 cm). As such, the length of the various nopalito cultivars ranged between 15.99 cm (Berg x Mexican) and 19.63 cm (Santa Rossa) with an average length of 17.20 cm. This means that Santa Rossa was the cultivar which had the longest nopalitos while Berg X Mexican had the shortest nopalitos on average. The majority of the nopalito cultivars did not differ significantly with regard to their length, however, Gymno Carpo and Berg X Mexican nopalitos were the shortest and differed significantly from Santa Rossa which had the longest nopalitos on average.

Width measures how wide an object is. From Table 6, one can deduce that the widest cultivar was Nudosa (11.26 cm) while the narrowest cultivar was Skinners Court (7.33 cm). The average of the cultivars amounted to 9.09 cm. Significant ($p < 0.001$) differences were observed among cultivars. In addition to Nudosa, the cultivars which ranged above the 10.00 cm mark, i.e., were wider than the rest of the cultivars, included Santa Rossa (10.23 cm), Fresno (10.48 cm) and Robusta (11.15 cm). Apart from Skinners Court, the narrowest cultivars included Zastron (7.82 cm), Messina (8.8 cm) and R1251 (8.28 cm). Skinners Court differed significantly from Nudosa and Robusta.

The minimal differences which were observed among the various cultivars suggest that the width of the stems may be a potential morphological marker, especially in situations where maximum precision is required. An example of such a situation is the industrial processing of cladodes, whereby the widest cladodes may exacerbate wastage.

The diameter of the nopalitos was the smallest measure of the size observed (Table 6), which also gave insight into the thickness of the stems. This parameter is a critical determinant of establishing which cultivars are of great significance because consumers prefer thin nopalitos. The measurements ranged between 0.42 cm (Santa Rossa) and 0.96 cm (Fusicaulis) with an average of 0.56 cm. Significant ($p <0.001$) differences were only observed in Fusicaulis (0.96 cm), Fresno (0.89 cm) and Nudosa (0.84 cm), which all had higher diameter values compared to the rest of the nopalito cultivars. These three aforementioned cultivars differed significantly from Santa Rossa.

3.4.1.3. Extracted mucilage yield and waste

The level of mucilage found in nopalitos is one of the attributes that consumers look for as it affects the eating qualities of the vegetable, where too high of a level of mucilage presents sliminess which is regarded as undesirable by many consumers (Calvo-Arriaga *et al*., 2010; Maki-Diaz *et al.,* 2015). Furthermore, such high values pose quite an encumbrance when extracting and liquefying cladodes (Stintzing & Carle, 2005). Significant ($p < 0.001$) differences were observed in the effects of cultivar on the pulp before centrifugation, extracted mucilage, waste, %Yield as well as %Waste (Table 6).

An important aspect to take into consideration during the extraction of mucilage is the waste that may occur in the process. Weighing the pulp of the various cultivars before the centrifugation process revealed significant ($p < 0.001$) differences among the cultivars in terms of pulp yield. The weight of the macerated and cooked nopalitos ranged between 32.79 g (R1251) and 129.00 g (Fusicaulis), with an average of 55.58 g. The heaviest pulps were obtained from Fusicaulis (129.00 g), Nudosa (112.86 g), Robusta (85.50 g), Santa Rossa (78.15 g) and Fresno (71.58 g). As expected, these aforementioned cultivars were recorded as the heaviest cultivars, when the nopalitos' weight was determined in this study. On the other side of the spectrum, the cultivars which measured the lowest macerated pulp weight were R1251 (32.79 g), Ofer (35.59 g), Morado (35.66 g) and Turpin (35.82 g) recorded the lowest weight when the nopalitos were weighed at the beginning of this study. An interesting observation was that the weight of the pulp of the macerated and cooked nopalitos before centrifugation, was not the same as their original weight. This implies that some of the moisture may have escaped from the nopalitos in the form of steam during the cooking process in the microwave; hence, the decreased weight measured from the pulp. It must be noted that the loss of some moisture from the cultivars does not imply that the nopalitos which had the lowest weight differences between the original pulp and the macerated cooked pulp, yielded the highest moisture content (Table 9).

The amount of mucilage which was extracted from the various nopalito cultivars ranged between 7.87 g (Ofer) and 69.63 g (Fusicaulis), with an average of 21.76 g which translates to a 20.84% average mucilage yield. Fusicaulis (69.63 g/42.27%), Nudosa (59.07 g/33.29%), Santa Rossa (37.29 g/27.24%), Fresno (34.23 g/ 26.42%) and Robusta (27.69 g/ 29.34%) yielded significantly ($p < 0.001$) higher mucilage contents compared to all of the cultivars of the study. On the other side of the spectrum, Ofer (7.87 g/11.31%), Malta (9.49 g/13.18%), Morado (9.54 g/14.54%) and Skinners Court (10.66 g/11.08%) all yielded significantly lower mucilage compared to the rest of the cultivars. This, in turn, implies that the four least mucilage-yielding cultivars (Ofer, Malta, Morado and Skinners Court) are a better option for consumption as they contain the least mucilage, which interferes with the eating qualities of the nopalitos. On the other hand, the five highest mucilage-yielding cultivars (Fresno, Fusicaulis, Nudosa, Santa Rossa and Robusta) would be the better option if the objective of processing requires maximum mucilage yields for various applications. The difference between the cultivar which yielded the lowest extracted mucilage (Ofer) in terms of weight (g) and the highest mucilage-yielding cultivar (Fusicaulis) was an 8.85-fold difference. One plausible explanation for such a big weight difference may be that the weight of the nopalitos may have influenced the yield (in grams) of mucilage that was extracted. This is because the likes of Fusicaulis recorded some of the heaviest nopalitos while Ofer recorded the least heavy nopalitos in the study. This is also evident in the rest of the heaviest cultivars, Fresno, Nudosa, Robusta and Santa Rossa, while the likes of Morado, Malta and Skinners Court followed a trend similar to that of Ofer.

Food has been noted to be wasted at various stages of the food production and supply chain by various stakeholders (FAO, 2011, Parfitt *et al*., 2010). With an estimated annual loss or waste of approximately one-third of the food produced for human consumption globally (FAO, 2011), it becomes important to measure the amount of waste as well as to identify the “hotspots of wastage” (Priefer *et al*., 2016). Significant ($p < 0.001$) differences were observed in the waste (remaining pulp after the extraction of mucilage) from the various cultivars, however, no significant differences were observed among the cultivars in terms of the %Waste obtained. The waste of the cultivars ranged between 23.26 g (Berg X Mexican) and 51.03 g (Fusicaulis), with an average of 31.65 g (37.90% of the original pulp before extraction of mucilage). The highest amount of waste was observed in Fusicaulis (51.03 g/38.07%), Nudosa (50.74 g/37.69%) and Fresno (48.71 g/26.10%), while the lowest amount was observed in Berg X Mexican (23.26 g/36.20%), R1251 (23.31 g/36.64%), Morado (23.75

g/40.32%) and Turpin (24.91 g/38.10%). When observing the %Waste of the various nopalito cultivars, one can deduce that the percentages which amounted to waste of the majority of the various cultivars ranged between 36 and 44% of the original pulp.

With the exception of Fusicaulis (38.07%) and Nudosa (37.69%), the percentage of waste which was observed in the heavier cladodes was low. In fact, it was relative to the %Yield of the mucilage which was extracted from these cladodes. For example, Santa Rossa whose mucilage yield amounted to 27.24%, recorded a %Waste of 28.90%. Robusta (29.34% mucilage yield) resulted in a %Waste of 29.51%, while Fresno (26.42% mucilage yield) recorded a %Waste of 26.10%. Though in the case of this study, the remaining pulp after the extraction of mucilage was regarded as waste, it must be noted that due to the cladodes' multifaceted nature, the “waste” can find application in other processes such as being incorporated into forage for livestock.

3.4.2. The effect of cultivar on the physical attributes of nopalitos

Table 7 shows the textural properties and viscosity of the 20 different nopalito cultivars.

Table 7: Analysis of variance (ANOVA) for the effect of cultivar on the various physical attributes of nopalitos harvested over two years

Cultivar	**Compressibility (mm)**	**Firmness (mm)**	**Viscosity (cP)**	**Line-spread (cm)**
Gymno Carpo	34.33 ± 13.96	68.67^{bc} ± 12.19	89.26 ±76.01	5.35^{cd}±0.49
R1251	38.08 ± 13.93	72.43^{bc}± 12.26	108.00±122.03	4.24^{ab}±0.47
Meyers	26.83± 14.87	64.00^{abc}± 13.58	103.25±108.37	5.12^{cd}±0.37
Skinners Court	30.18 ± 18.87	59.75^{abc}± 18.16	34.80±16.77	3.99^{a}±0.42
Algerian	50.67 ± 12.79	75.26^{c}± 6.88	181.54±154.72	4.17^{ab}±0.68
Ofer	34.75± 20.95	69.26^{bc}± 9.00	67.52±79.06	4.15^{ab}±0.40
Turpin	37.00± 18.93	66.50^{bc}± 8.89	154.91±151.74	5.16^{cd}±0.46
Messina	40.25± 17.16	64.38^{abc}± 15.03	186.45±158.73	4.16^{ab}±0.46
Santa Rossa	36.25± 14.28	61.31^{abc}± 14.43	100.35±100.88	4.61^{abcd}±0.66
Berg X Mexican	32.56± 21.38	65.85^{bc}± 6.91	111.41±115.00	4.88^{bcd}±0.76
R1260	38.25± 19.71	64.88^{abc}± 14.13	62.91±56.98	5.22^{cd}±0.89
Fresno	26.64±18.87	51.17^{ab}±18.65	132.42±118.94	5.35^{d}±0.78
Fusicaulis	37.33±21.63	55.53^{abc}±17.22	51.59±19.31	4.83^{abcd}±0.68
Malta	37.92±20.86	64.35^{abc}±11.73	106.05±96.96	3.99^{a}±0.29
Morado	38.00±20.28	67.82^{bc}±10.66	73.40±64.80	4.81^{abcd}±0.82
Nudosa	40.33±11.68	41.94^{a}±27.18	113.33±111.28	4.87^{bcd}±0.79
Robusta	38.82±18.61	50.72^{ab}±31.86	43.93±43.52	4.67^{abcd}±0.33
Zastron	34.83±18.02	61.53^{abc}±10.35	170.46±159.26	4.66^{abcd}±0.45
Rossa	35.67±10.03	61.93^{abc}±19.49	91.89±78.29	4.49^{abc}±0.49
Sicilian Indian Fig	38.50±24.14	66.58^{bc}±15.77	109.26±87.02	4.53^{abcd}±0.43
Average	36.36	62.69	100.14	4.61
Sign level	$p = 0.468$	$p < 0.001$	$p = 0.061$	$p < 0.001$

Means with different superscripts in the same column differ significantly

3.4.2.2. Viscosity of the extracted mucilage

The mucilage found in cladodes is known to exhibit high viscosity which, in turn, makes it a popular hydrocolloid in the food industry to incorporate into products like beverages and salad dressings to offer viscosity. Brookfield Engineering Laboratories (2014) has described the viscosity of a fluid as the physical manifestation of the molecular weight and the distribution of molecules. There are various methods in which viscosity can be measured, however, Du Toit (2017), found that the line-spread method provided the most quantifiable data.

When looking at the results in this study, the above observation by Du Toit proves to be true, seemingly because significant ($p < 0.001$) differences were only identified in the average viscosity readings of the cultivars when determined using the line-spread method as opposed to the viscometer (Table 7). Albeit, no significant ($p = 0.061$) differences were observed in the results obtained using the viscometer, the average viscosity readings of the cultivars ranged between 34.80 cP (Skinners Court) and 186.45 cP (Messina), with an average of 100.14 cP. The majority of the cultivars were highly resistant to flow (i.e., highly viscous), with the likes of Messina (186.45 cP), Algerian (181.54 cP), Zastron (170.46 cP), Turpin (154.91 cP) and Fresno (132.42 cP) being the most viscous cultivars. Nudosa (113.33 cP), Berg X Mexican (111.41 cP), Sicilian Indian Fig (109.26 cP), R1251 (108.00 cP), Malta (106.05 cP), Meyers (103.25 cP) and Santa Rossa (100.355 cP), were also more viscous compared to the rest of the nopalito cultivars. The conspicuous range among the cultivars is evidence of the diversity among the cultivars in terms of viscosity.

The line-spread test provides a simple and economical way of determining the viscosity of fluids (Mann & Wong, 1996). Nicosia and Robbins (2007) have further suggested that the values obtained from this test, also determine the surface tension as well as the density of the fluid under investigation.

The viscosity of the various cultivars using the line-spread test ranged between 3.99 cm (Skinners Court and Malta) and 5.35 cm (Gymno Carpo and Fresno), with an average of 4.61 cm (Table 7). Significant ($p < 0.001$) differences were observed among the different cultivars. Gymno Carpo (5.35 cm), Fresno (5.35 cm), R1260 (5.22 cm), Turpin (5.16 cm) and Meyers (5.12 cm), all travelled significantly the furthest across the surface as compared to the other nopalito cultivars. This implies that the aforementioned cultivars are the least viscous in the study. Skinners Court (3.99 cm), Malta (3.99 cm), Ofer (4.15 cm), Messina (4.16 cm) and Algerian (4.17 cm), travelled the shortest distance across the surface, making them the most viscous cultivars in this test. This, in turn, means that Skinners Court, Malta, Messina and Algerian would provide the greatest viscosity to products that they are added into at the least concentrations.

3.4.2.3. Colour

In Table 8, the colour attributes of the 20 different nopalito cultivars are observed.

Table 8: Analysis of variance (ANOVA) for the effect of cultivar on the various colour attributes of nopalitos harvested over two years

Cultivar	L*	a*	b*	Chroma (Saturation Index)	Hue Angle
Gymno Carpo	53.38^{c}±3.26	-20.16^{a}±1.75	34.30^{c}±5.10	39.81^{c}±5.23	-59.35^{ab}±1.95
R1251	52.35^{bc}±2.40	-19.64^{ab}±1.03	32.13^{bc}±3.54	37.67^{bc}±3.48	-58.43^{abcd}±1.84
Meyers	52.39^{bc}±3.61	-19.53^{ab}±4.73	33.56^{c}±7.41	38.88^{c}±8.50	-59.12^{abc}±2.24
Skinners Court	49.26^{abc}±2.40	-17.94^{ab}±3.01	29.14^{abc}±6.60	34.26^{abc}±7.06	-57.91^{abcd}±3.07
Algerian	48.50^{abc}±5.72	-15.91^{ab}±4.71	24.57^{abc}±10.36	29.33^{abc}±11.22	-55.67^{abcd}±4.14
Ofer	50.12^{abc}±4.23	-17.11^{ab}±4.15	27.20^{abc}±9.00	32.17^{abc}±9.78	-56.99^{abcd}±3.24
Turpin	52.74^{c}±3.05	-19.25^{ab}±1.59	32.37^{bc}±4.29	37.68^{bc}±4.40	-59.13^{abc}±1.78
Messina	51.42^{abc}±2.72	-19.17^{ab}±1.52	32.57^{bc}±3.93	37.80^{bc}±4.08	-59.40^{ab}±1.59
Santa Rossa	52.27^{bc}±3.59	-18.32^{ab}±3.59	31.40^{abc}±5.32	35.98^{abc}±5.88	-58.60^{abcd}±1.79
Berg X Mexican	48.46^{abc}±6.36	-17.16^{ab}±5.76	28.13^{abc}±11.52	33.02^{abc}±12.68	-57.49^{abcd}±5.19
R1260	51.49^{abc}±4.75	-19.69^{ab}±1.71	32.84^{c}±4.76	38.31^{bc}±4.89	-58.87^{abc}±1.87
Fresno	46.31^{ab}±3.92	-14.30^{b}±4.37	20.65^{a}±8.39	25.15^{a}±9.35	-54.41^{cd}±3.12
Fusicaulis	45.33^{a}±3.54	-14.12^{b}±4.74	20.70^{a}±6.90	25.13^{a}±8.12	-56.09^{abcd}±5.55
Malta	49.73^{abc}±5.19	-15.45^{ab}±5.89	24.99^{abc}±11.88	28.26^{abc}±13.96	-53.99^{d}±6.10
Morado	49.83^{abc}±6.22	-18.30^{ab}±4.21	28.17^{abc}±9.87	33.86^{abc}±9.76	-58.02^{abcd}±2.71
Nudosa	48.65^{abc}±3.85	-17.20^{ab}±3.96	26.24^{abc}±8.38	31.41^{abc}±9.14	-55.99^{abcd}±3.09
Robusta	47.34^{abc}±3.95	-14.95^{ab}±4.55	21.67^{ab}±8.24	26.37^{ab}±9.28	-54.64^{bcd}±3.80
Zastron	49.72^{abc}±2.97	-17.71^{ab}±4.13	28.50^{abc}±6.08	33.57^{abc}±7.26	-58.45^{abcd}±2.70
Rossa	52.12^{bc}±4.50	-18.20^{ab}±4.17	31.07^{abc}±7.62	36.03^{abc}±8.59	-59.63^{a}±2.17
Sicillian Indian Fig	50.18^{abc}±4.95	-17.40^{ab}±3.62	27.90^{abc}±5.17	32.90^{abc}±6.18	-58.31^{abcd}±2.81
Average	50.08	-17.58	28.41	33.38	-57.52
Sign. Level	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

Means with different superscripts in the same column differ significantly

The colour of fruits and vegetables is one of the most critical attributes as it may assist in determining the health and ripeness of the crop. Furthermore, the colour may assist in determining the kind of nutrients which are present in those fruits and vegetables (Bruso, 2018). Green vegetables such as nopalitos are green in colour as a result of the chlorophyll present in them. Nutrients which are common in such coloured vegetables include Vitamin A, Vitamin C and lutein, which is important for healthy vision (Bruso, 2018). Significant ($p < 0.001$) differences in colour properties (L*, a*, b*, C* and hue values) were observed in the effect of cultivar on the various colour properties (Table 8). The L* coordinates which measured the lightness of the colour of the cladodes ranged between 45.33 (Fusicaulis) and 53.38 (Gymno Carpo) with a lightness average of 50.08. Gymno Carpo (53.38) and Turpin (52.74) had significantly higher values of lightness compared to the other 18 nopalito cultivars. In comparison, Fusicaulis (45.33) and Fresno (46.31) recorded significantly ($p < 0.001$) lower values, compared to the rest of the nopalito cultivars.

Ramírez-Moreno *et al*. (2013) reported on the L* values of raw "Atlixco' (64.67) and 'Milpa Alta' (65.21) cladode cultivars. From the results obtained for those two cultivars, one can deduce that the 20 nopalito cultivars which were observed in this study were less light than those previously reported by Ramríez-Moreno *et al.* (2013).

Significant ($p < 0.001$) differences were observed in the degree of greenness (a*) of the various cultivars. The brightest green colour was observed in Gymno Carpo (-20.16) while the darkest shades of green were observed in Fusicaulis (-14.12) and Fresno (-14.30). Apart from the three aforementioned cultivars, no significant differences were observed in the rest of the 17 cultivars. Ramírez-Moreno *et al*. (2013) reported on the a* values of raw "Atlixco' (-10.45) and 'Milpa Alta' (-10.11) cladode cultivars. From the results obtained for those two cultivars, one can deduce that the 20 nopalito cultivars which were observed in this study were much greener. Furthermore, all a* values that were recorded fell below the 0 mark (i.e., were all negative values), which indicates that the nopalitos had not succumbed to chlorophyll degradation at the time of harvest which would, in turn, have resulted in a more red colour.

Significant ($p < 0.001$) differences were observed in the degree of yellowness (b* values) between the various cultivars. The average b* value of the various cultivars was 28.41. The greatest intensity of yellow was observed in Gymno Carpo (34.30), Meyers (33.56) and R1260 (32.84), while Fresno (20.65) and Fusicaulis (20.70) recorded the lowest intensities. The majority of the nopalito cultivars did not differ significantly with regard to their degree of yellowness, with many of them having measured between 24 and 31. Ramírez-Moreno *et al.* (2013) reported on the b* values of raw "Atlixco' (17.36) and 'Milpa Alta' (18.10) cladode cultivars. From the results obtained for those two cultivars, one can deduce that the 20 nopalito cultivars which were observed in this study had more yellow intensity than those reported by Ramírez-Moreno *et al*. (2013). One interesting observation that was made in this study was the result of Robusta (21.67). This cultivar is known to be blue-green in colour and contains blue undertones. Because of this, one would naturally expect its b* value to be the closest to 0 or even below zero. This proves that the perception of colour through eye observation cannot be used to determine such attributes. This also implies that Fresno, Fusicaulis and Robusta are closely similar in terms of their degree of yellowness.

Chroma measurements depict the extent to which the tone is pale or intense (Meraz-Maldonado *et al.,* 2012*)*. Furthermore, Meraz-Maldonado *et al.* (2012) have associated it with brightness. The aforementioned statement is supported by the observed results in the effect of cultivar on the various properties, which had significant ($p < 0.001$) differences. Gymno Carpo, which yielded the highest L* value, also yielded the highest chroma value (39.81). On the other side of the spectrum, Fusicaulis, which yielded the lowest L* value also yielded the lowest chroma value (25.13). Another pair of cultivars which supports the aforementioned observation by Meraz-Maldonado *et al.* (2012) is Meyers (38.88) and Fresno (25.15). This, in turn, implies that Gymno Carpo and Meyers have a greater intensity of green, while Fusicaulis and Fresno are the palest. Cultivars R1251 (37.67) and Turpin (37.68) also had high intensities of green as compared to all the other cultivars. The majority of the cultivars were not significantly different with regard to their Chroma values.

Hue is used for the classification of colours and is responsible for forming the colour wheel (Konica Minolta, 2007). In terms of the effect of cultivar on the various attributes, the hue angles ranged between -53.99 (Malta) and -59.63 (Rossa) with an average hue value of -57.52. Significant ($p <0.001$) differences were observed among the cultivars. All hue angles obtained from the nopalito cultivars were negative, and when one observes Figure 6, one can deduce that the results obtained in this study, fall in the second quadrant counterclockwise, where a* values are negative and b* values are positive. The quadrant and range in which all 20 cultivars fall (between -50 and -60), implies that their hues depict a yellow-green colour, with the likes of Rossa (-59.63), Messina (-59.40), Gymno Carpo (-59.35), Turpin (-59.13) and Meyers (-59.12) having recorded the highest saturation (intensity) of green, while Malta (-53.99), Fresno (-54.41) and Robusta (-54.64) had the lowest saturation of green. The majority of the cultivars were not significantly different with regard to their hue values. Du Toit *et al.,* (2017) also reported the presence of yellow-green hues in mucilage that was obtained from mature cladodes.

The investigation of the colour properties of the nopalitos has revealed that the 20 cultivars are of high quality. The luminosity (L*) indicated that the brightness of the nopalitos was medium (average 50.08). The a* values of the cultivars averaged -17.58 and when observing Figure 6, one can deduce that it implies that the values are in the direction of the colour green as opposed to that of red. The b* values (average 28.41) indicated that the cultivars possessed a yellow undertone as opposed to a blue one.

The Chroma (C*) values (average 33.38) observed, illustrated the high intensity of tones, while the mean h˚ (-57.52) indicated the cultivars to be yellow-green.

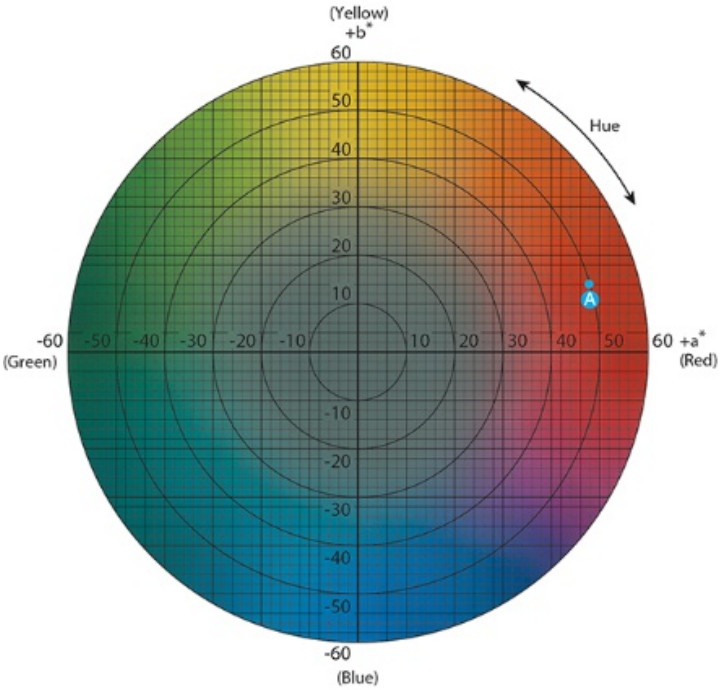

*Figure 6: L*a*b colour space chromaticity diagram (Hue and Saturation (www.konicaminolta.com, 2007)*

3.4.3. The effect of cultivar on the physico-chemical and chemical attributes of nopalitos

Table 9, shows the effect of cultivar on the various physico-chemical characteristics of the 20 nopalito cultivars.

Table 9: Analysis of variance (ANOVA) for the effect of cultivar on the various physico-chemical and chemical attributes of nopalitos harvested over two years

Cultivar	Moisture content (%)	% Solids	pH	%Titratable acidity expressed as citric acid	Sugar (TSS Brix°)
Gymno Carpo	77.51 ± 21.72	22.49 ± 21.72	4.22 ± 0.24	0.38^{bc}±0.17	5.77^{abcde}±0.77
R1251	84.20 ± 11.34	15.80± 11.34	4.21± 0.26	0.42^{bc}±0.09	6.98^{ef}±1.15
Meyers	91.74± 10.91	8.26± 10.91	4.21± 0.24	0.40^{bc}±0.09	5.37^{abc}±0.33
Skinners Court	85.30 ± 4.50	14.70 ± 4.50	4.21 ± 0.13	0.15^{a}±0.05	5.72^{abcde}±1.29
Algerian	90.08 ± 2.85	9.92 ± 2.85	4.13 ± 0.22	0.42^{bc}±0.03	5.64^{abcd}±0.86
Ofer	84.27± 8.76	15.73± 8.76	4.19± 0.21	0.36^{b}±0.12	5.74^{abcde}±0.11
Turpin	91.49± 27.37	8.51± 27.37	4.02± 0.17	0.57^{c}±0.09	7.32^{f}±1.10
Messina	85.17± 12.14	14.83± 12.14	4.25± 0.19	0.44^{bc}±0.13	6.02^{abcdef}±0.58
Santa Rossa	86.25± 38.25	13.75± 38.25	4.18± 0.18	0.31^{ab}±0.15	6.15^{abcdef}±1.32
Berg X Mexican	88.23± 2.17	11.77± 2.17	4.01± 0.09	0.38^{bc}±0.11	6.67^{cdef}±0.98
R1260	89.31± 3.71	10.69± 3.71	4.11± 0.25	0.28^{ab}±0.04	6.27^{bcdef}±0.72

Fresno	83.70±39.49	16.30±39.49	4.15±0.22	0.37^{b}±0.17	6.35^{bcdef}±1.29
Fusicaulis	87.93±1.46	12.07±1.46	4.17±0.22	0.30^{ab}±0.10	5.20^{ab}±1.12
Malta	88.99±2.51	11.01±2.51	4.20±0.20	0.38^{b}±0.11	5.73^{abcde}±0.93
Morado	86.01±9.10	13.99±9.10	4.13±0.27	0.33^{ab}±0.18	6.25^{bcdef}±0.65
Nudosa	86.37±3.31	13.63±3.31	4.12±0.17	0.29^{ab}±0.16	4.89^{a}±0.36
Robusta	87.21±3.56	12.79±3.56	4.21±0.19	0.35^{b}±0.16	5.95^{abcde}±1.02
Zastron	85.51±3.60	14.49±3.60	4.14±0.23	0.37^{b}±0.13	6.75^{def}±0.58
Rossa	85.53±4.41	14.47±4.41	4.18±0.26	0.38^{b}±0.18	6.66^{cdef}±0.70
Sicillian Indian Fig	86.69±2.98	13.31±2.98	4.08±0.27	0.42^{bc}±0.16	5.98^{abcde}±0.81
Average	86.59	13.43	4.16	0.37	6.07
Sign. Level	$p = 0.966$	$p = 0.966$	$p = 0.389$	$p < 0.001$	$p < 0.001$

Means with different superscripts in the same column differ significantly

3.4.2.1. Compressibility and firmness

Compressibility measures how soft or hard plant tissue is. High values of this attribute indicate nopalitos which have much more soft tissue while low values indicate nopalitos which have hard tissue. No significant differences were observed among the nopalito cultivars, whose compressibility ranged between 26.64 mm (Fresno) and 50.67 mm (Algerian), with an average of 36.36 mm (Table 7).

Albeit, no significant ($p = 0.468$) differences were observed, one can say that the plant tissues of Fresno (26.64 mm) and Meyers (26.83 mm) were the hardest as they showed the lowest measures of compressibility. Skinners Court (30.18 mm) and Berg X Mexican (32.56 mm) had plant tissue that was softer than Fresno and Meyers, however, it was a little harder than the majority of the other cultivars. On the other side of the spectrum, Algerian (50.67 mm), Nudosa (40.33 mm) and Messina (40.45 mm) had the highest measures of compressibility, meaning that their plant tissues were the softest. The majority of the cultivars ranged between 34 and 38 mm.

Firmness indicates how flexible the nopalitos are. High values indicate thin and flexible nopalitos while low values indicate firm nopalitos. Significant ($p < 0.001$) differences in this attribute were observed in the effects of cultivar on this attribute, with an average of 62.69 mm (Table 7). The thinnest and thus, most flexible cultivar was Algerian (75.26 mm), R1251 (72.43 mm), Ofer (69.26 mm), Gymno Carpo (68.67 mm), Morado (67.82 mm), Sicilian Indian Fig (66.58 mm), Turpin (66.50 mm) and Berg X Mexican (65.85 mm) were also more flexible than the rest of the nopalito cultivars. The least flexible and thus the thickest cultivars were Nudosa

(41.94 mm), Robusta (50.72 mm) and Fresno (51.17 mm). The remaining cultivars' firmness ranged between 55 and 64 mm, with no significant differences observed.

3.4.3.1. pH

pH measures the acidity or basicity of a substance. According to Cantwell *et al*. (1992), the acidity of nopalitos is most likely to be approximately 0.94% in the morning while it is most likely to reduce as the day progresses to approximately 0.47% in relation to the exposure to light. As such, it has been recommended by Pimentel-González (2013) to harvest nopalitos two hours after sunrise, because it is at this time that the acid content is still low. No significant (p = 0.389) differences were observed in the effect of cultivar on pH (Table 9). The pH of the various cultivars ranged between 4.01 and 4.25, with an average of 4.16. Rodríguez-Garcia *et al.* (2007) reported on the pH of fresh and powdered nopalitos of the *Opuntia ficus-indica* Redonda variety, which weighed between 60-200 g. From their observations, the pH of the fresh nopalitos ranged between 4.14 and 4.41, while the powdered nopalitos ranged between 4.07 and 4.35. The pH of the twenty South African nopalito cultivars of this study, falls well within the range of the nopalitos which were reported by Rodríguez-Garcia *et al*. (2007). Albeit, no significant differences were observed, one can deduce that Messina (4.25) and Gymno Carpo (4.22) had the lowest acidity, while Berg X Mexican (4.01) and Turpin (4.02) recorded the highest acidity. This, in turn, implies that Berg X Mexican and Turpin are most likely to be the most sour cultivars compared to the rest of the cultivars as a result of the two cultivars' high acidity. The different levels of acidity found in nopalitos are known to greatly affect the gustatory qualities of the vegetable, with the majority of consumers having a greater preference for cultivars which have the lowest levels of acidity (Maki-Díaz *et al.,* 2015). From a technical perspective, Messina and Gymno Carpo would be better preferred cultivars over Berg X Mexican and Turpin, because low acidity is a desirable trait in the eating qualities of nopalitos. However, in this case, the differences were so minute that the majority of consumers wouldn't necessarily detect the differences in sourness.

3.4.3.2. Moisture and %solids

Moisture content is a common method of measurement during food processing as well as testing (Canet, 1988). It is a parameter of paramount importance to food manufacturers as well as consumers (Canet, 1988). The moisture content of fruits and vegetables varies significantly with raw cucumbers (96%) and melons (92 – 94%) having high contents of water, while green lima beans (67%) and avocadoes (65%) have lower water contents. Nopalitos have been reported to be high in moisture, which in turn gives insight into their high level of turgidity which is a desirable trait (Stintzing & Carle, 2005). Turgidity refers to the swelling of tissue or cells as a result of water uptake (Lane *et al.,* 2017). Rodríguez Félix and Cantwell (1988) as well as Meraz-Maldonado *et al.* (2012) have reported that the development of the parenchyma in nopalitos often leads to an increase in water storage capacity as well as the moisture content.

The moisture content of the 20 nopalito cultivars ranged between 77.51% (Gymno Carpo) and 91.74% (Meyers), with an average of 86.59% (Table 9). No significant (p = 0.966) differences were observed among the various nopalito cultivars. Though the differences were inconspicuous, Meyers (91.74%), Turpin (91.49%), Algerian (90.08%) and R1260 (89.31%) recorded the highest moisture content, while Gymno Carpo (77.51%), Fresno (83.70%), R1251 (84.20%) and Ofer (84.27%) recorded lower contents of moisture. The majority of the cultivars recorded a moisture content between 85 and 88%. Rodríguez-Felix and Cantwell (1988) investigated cladodes from the *O. amyclaea*, *O. ficus-indica O. inermis* whose moisture content amounted to 92.0%, 91.5% and 91.7%, respectively. This proves, just how high the moisture content found in nopalitos is.

Soluble solids refer to the acids and sugars which are found in plants, together with minuscule amounts of dissolved fructans, minerals, phenolic compounds, pigments, proteins, as well as vitamins (Tadeo *et al*., 1987; Ito *et al*.,1997; Chope *et al*., 2006; Kader, 1999, 2008a,b). Total soluble solids are a critical quality parameter used to signify how sweet fresh and processed horticultural food products are, and assist in research as well as determining marketing standards in laboratories and the industry, respectively (Magwaza and Opara, 2015). No significant (p = 0.966) differences were observed among the cultivars, which ranged between 8.26% (Meyers) and 22.49% (Gymno Carpo), with an average of 13.43% (Table 9). Meyers (8.26%), Turpin (8.51%), Algerian (9.92%) and R1260 (10.69%) had the least amount of soluble solids. The majority of the nopalitos measured total soluble solids between 11 and 16%. Mogwaza and Opara (2015) have noted that the concentration of soluble solids in fruits and vegetables is not static and instead varies during maturation and ripening.

Betancourt-Domínguez *et al.* (2006) reported on the cladodes of the *Opuntia* Blanco sin Espinas, Blanco con Espinas and Verde Valtierrilla varieties, with the size range of 14 cm, 17 cm and 22 cm. In the study, the authors observed the highest amount of total soluble solids at the smallest length of nopalitos (14 cm). On the other side of the spectrum, Rodríguez-Felix and Cantwell (1988) reported an increase in total soluble solids at larger sizes. This implies that the total soluble solids of nopalitos are independent of the variety and size as suggested by Betancourt-Dominguez *et al.* (2006).

3.4.3.3. Sugar content (TSS, °Brix)

°Brix sugar measurements are a good indication of the status of a plant's health, sugar content, as well as its mineral content (Invisible Gardener, 2019). High readings of this attribute signify a high level of complex carbohydrates and dissolved solids (Invisible

Gardener, 2019). Significant ($p < 0.001$) differences in this attribute were observed in the effect of cultivar on the various properties. The sugar readings of the various nopalito cultivars ranged between 4.89 °Brix (Nudosa) and 7.32 °Brix (Turpin), with an average of 6.07 (Table 9). Turpin (7.32 °Brix), R1251 (6.98 °Brix), Zastron (6.75 °Brix), Berg X Mexican (6.67 °Brix), Rossa (6.66 °Brix), Fresno (6.35 °Brix), R1260 (6.27 °Brix) and Morado (6.24 °Brix) all had relatively higher readings compared to the rest of the nopalitos. Nudosa, on the other hand, had the least °Brix sugar content, while the rest of the nopalito cultivars were average. The results obtained in this study reveal just how low the sugar content of nopalitos is, as opposed to cactus pear fruits whose juice can record a °Brix reading of 16.5 up to 60 °Brix (Sáenz *et al.,* 1998).

3.4.3.4. Titratable acidity

The level of acidity found in nopalitos affects the eating qualities of the vegetables. Too high of a level of acidity presents sourness which is often regarded as undesirable by consumers. Titratable acidity is an important parameter in the determination of organic acids present in nopalitos. Jianqin *et al.* (2002) have reported that cladodes primarily comprise various acids such as citric, malic and oxalic acid which are all affected by climate, soil and storage conditions (Cantwell *et al.,* 1992). Significant ($p < 0.001$) differences were observed in the effect of cultivar on this attribute. The titratable acidity ranged between 0.15% expressed as citric acid (Skinners Court) and 0.57% expressed as citric acid (Turpin), with an average of 0.37% expressed as citric acid (Table 9). Turpin (0.57% expressed as citric acid), Messina (0.44% expressed as citric acid), R1251 (0.42% expressed as citric acid), Sicilian Fig Indian (0.42% expressed as citric acid) and Meyers (0.40% expressed as citric acid) recorded the highest amounts of organic acids. On the other side of the spectrum, Skinners Court (0.15% expressed as citric acid) was the least organic acid-containing cultivar while the rest of the cultivars had average values. All values which were observed in this study fall within the ranges which have been reported by various researchers who have noted the titratable acidity of nopalitos to range between 0.12% and 0.87% of citric acid (Villarreal *et al.,* 1963; Rodríguez-Félix and Cantwell, 1988).

3.4.4. The effect of the year of harvest on the various nopalito attributes

Seasonality refers to the regular change in the environment as well as the biological responses acclimatised by that environment (Battey, 2000). Cactus pear plants are well adapted to grow and survive in the harshest of conditions where there is immense water stress and poor soil. Nopalitos are regarded as an important re-vegetation crop to regulate water and erosion in areas which are under environmental duress (Pimienta, 1994). It has been noted that in Mexico, flowers of the *O. ficus-indica* species develop from as early as March and end in late April; the fruits develop from mid-April to late August, while the stem growth occurs between

April and July of every year (Pimienta *et al.,* 2000). The stem extension period occurs from mid-September through to December, while the growth and differentiation of fine roots fall in with the summer rainy season between July and September (Pimienta *et al.,* 2000). Because South Africa is in the southern hemisphere of the world, the cycle of the cactus plants is opposite to that of the ones which are found in Mexico, with cactus fruit coming into season in the summer, from early January to the middle of March and nopalitos being collected between the spring and summer period (October to November). Differences in climatic and environmental conditions have a big impact on the quality and yield of plants in each year's harvest. As such, it becomes important to investigate the extent to which these aspects affect the quality of these plants. Tables 10, 11 and 12 show the effects of the year of harvest on the various attributes of the nopalitos. From these tables, only the attributes which have significant differences will be discussed below. No significant differences were observed in the effects of year of harvest on the weight ($p = 0.887$), length ($p = 0.981$), width ($p = 1.141$), diameter ($p = 0.915$), moisture content ($p = 0.730$), % solids ($p = 0.730$), measurement of viscosity using the line-spread method (0.945) as well as the sugar content (TSS °Brix ($p = 0.055$)) of the nopalito cultivars.

3.4.4.1. The effect of year of harvest on the morphological and physiological attributes of nopalitos

Table 10 shows the means of morphological characteristics and the yield of pulp and mucilage data from the 20 nopalito cultivars over two years.

Table 10: Analysis of variance (ANOVA) for the influence of year of harvest on the morphological and physiological attributes of nopalitos

	2018	2019	Sign level
Weight (g)	88.54 ± 41.72	89.37 ± 49.54	$p = 0.887$
Length (cm)	17.21 ± 2.17	17.20 ± 2.91	$p = 0.981$
Width (cm)	8.92 ± 1.60	9.25 ± 1.87	$p = 0.141$
Diameter (cm)	0.55 ± 0.23	0.56 ± 0.23	$p = 0.915$
Pulp (before centrifuge) (g)	44.06^a ± 34.67	67.09^b ± 41.48	$p < 0.001$
Extracted mucilage (g)	17.18^a ± 20.43	26.34^b ± 27.41	$p = 0.004$
Waste (g)	24.09^a ± 13.22	39.23^b ± 17.86	$p < 0.001$
% Mucilage Yield	16.43^a ± 12.64	25.25^b ± 14.15	$p < 0.001$
% Waste	28.03^a ± 10.74	47.77^b ± 11.59	$p < 0.001$

Means with different superscripts in the same row differ significantly

A. *Extracted mucilage yield and waste*

The Cactaceae family of plants owe much of their ability to retain water under stressful weather conditions to the mucilage that they contain (Sáenz *et al.,* 2004; Gebresamuel and Gebre-Mariam, 2012). The composition of the mucilage is known to vary among the species as well as the areas in which they grow (Sáenz *et al*., 2004; Gebresamuel and Gebre-Mariam, 2012). Significant ($p = 0.004$) differences were observed in the amount of mucilage that was extracted in the two years (Table 10). A higher yield was observed in 2019 (26.34 g/ 25.25% yield) as compared to 2018 (17.18 g/16.43% yield) (Figure 7). This may be attributed to the soil moisture content of the area in which the cultivars were grown. It has been reported that a linear relationship exists between the amount of mucilage that is extracted as well as the moisture content due to the heteropolysaccharide components found in cladodes, which have the ability to absorb water (Monrroy *et al.,* 2017).

The waste that occurred in the extraction of mucilage from the nopalito cultivars was over half of the original pulps before extraction in both years. The waste observed may have come as a result of the preparatory phase of the study where the outer parts of the stems were trimmed. Significant ($p < 0.001$) differences were observed in the weight of the original pulp that was obtained from the nopalito cultivars over the two years (Table 10). A much higher pulp content was observed in 2019 (67.07 g) than in 2018 (44.06 g).

The amount of waste was higher in 2019 (39.23 g/47.77% waste) as opposed to 2018 (24.09 g/28.03% waste) (Table 10 and Figure 7). Because the amount of mucilage found in nopalitos directly affects the eating quality of nopalitos, with high amounts of mucilage presenting an undesirable slimy texture, one can say that the 2018 nopalitos were better in this aspect due to the low mucilage that was present.

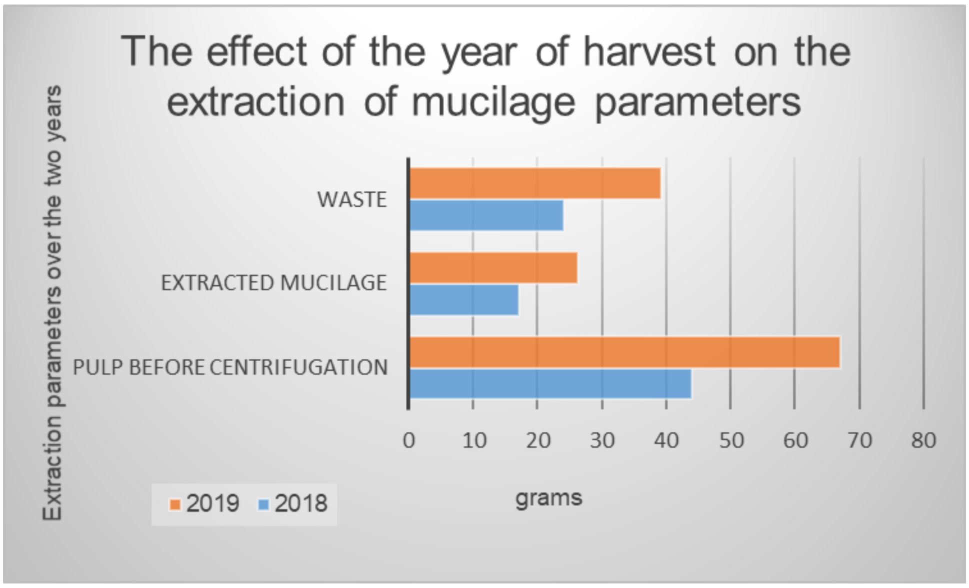

Figure 7: The effect of the year of harvest on the extraction of mucilage parameters obtained from the various nopalito cultivars over the two years

3.4.4.2. The effect of year of harvest on the physical attributes of nopalitos

Table 11 shows the means of physical attributes data of the 20 nopalito cultivars over two years.

Table 11: Analysis of variance (ANOVA) for the influence of year of harvest on the physical attributes of nopalitos

	2018	2019	Sign level
L*	$52.18^{b} \pm 3.99$	$47.98^{a} \pm 4.18$	$p < 0.001$
a*	$-19.17^{a} \pm 3.03$	$-15.98^{b} \pm 4.52$	$p < 0.001$
b*	$31.74^{b} \pm 7.42$	$25.07^{a} \pm 8.02$	$p < 0.001$
Chroma (Saturation Index)	$37.08^{b} \pm 7.81$	$29.68^{a} \pm 9.10$	$p < 0.001$
Hue Angle	$-58.22^{a} \pm 3.15$	$-56.83^{b} \pm 3.91$	$p = 0.003$
Compressibility (mm)	$24.26^{a} \pm 12.00$	$48.46^{b} \pm 14.36$	$p < 0.001$
Firmness (mm)	$55.87^{a} \pm 13.17$	$69.52^{b} \pm 17.94$	$p < 0.001$
Viscosity (cP)	$22.98^{a} \pm 13.87$	$186.30^{b} \pm 101.40$	$p < 0.001$
Line-spread (cm)	4.66 ± 0.74	4.67 ± 0.67	$p = 0.945$

Means with different superscripts in the same row differ significantly

B. Colour

The colour of nopalitos is perceived to be green, however, a considerable variation exists in the various cultivars' intensity of this attribute. Nobel (2001) has reported that there are specific cladode cultivars which exhibit a pale green colour. Furthermore, it has been reported that the chlorophyll content and environmental aspects such as water availability, micro- and macro-nutrients and irradiance are the main determinants of colour in nopalitos (Irizar-Garza and

Peña-Valdivia 2000; Nobel, 2001). Significant differences were observed in the different colour coordinates of the nopalitos over the two years (Table 11). The 2018 harvest (52.18) produced nopalitos which had significantly ($p < 0.001$) lighter (L*) nopalitos compared to those produced in 2019 (47.98). The a* values of the nopalitos which were produced in 2019 (-15.98) had a significantly ($p < 0.001$) less green tone than those produced in 2018 (-19.17). The b* values of the nopalitos which were observed in 2018 (31.74) were significantly ($p < 0.001$) higher and thus had a more yellow tone than those observed in 2019 (25.07). The saturation index (C*) of the nopalitos which were harvested in 2019 (29.68) was significantly ($p < 0.001$) lower and thus less bright than those which were harvested in 2018 (37.08). With regards to the hue angles (h°) that were observed in the two years, the nopalitos which were harvested in 2018 (-58.22) had significantly ($p = 0.003$) lower hues which translate to higher intensities of the green colour as compared to the nopalitos that were harvested in 2019 (-56.83). From these results which were obtained from the colour attributes of the nopalitos, one can deduce that 2018 generally produced nopalitos of a higher colour standard than 2019. The differences in colour quality between the two years may be ascribed to the environmental temperatures that were recorded. Since an increase in temperature to a certain extent, increases the rates of photosynthesis, it may in turn explain the colour quality differences that were observed among the nopalito cultivars over the two years, as 2018 (25.66°C) recorded higher average environmental temperatures as compared to 2019 (22.98°C).

C. Compressibility and firmness

Measuring the compressibility and firmness of nopalitos is an important aspect of profiling the textural properties of the various cultivars. By so doing, consumers can be assured of high-quality nopalitos which are thin, crispy and turgid. Significant ($p < 0.001$) differences were observed in the compressibility and firmness of the nopalitos that were harvested in 2018 and 2019 (Table 11). The 2019 harvest (48.46 mm) produced nopalitos which possessed much softer tissue compared to those produced in 2018 (24.26 mm). Because nopalitos need not be too hard nor too soft, one can say that the compressibility that was observed in 2019 was better as compared to the compressibility measured in 2018. With regards to the firmness of the nopalitos that was measured over the two years, one can deduce that the nopalitos which were harvested in 2018 (55.87 mm) were less flexible than those produced in 2019 (69.52 mm).

D. Viscosity of the extracted mucilage

The viscosity of mucilage may be affected by certain parameters. Du Toit *et al.* (2019), have reported the effects of temperature on the viscosity of mucilage under the investigation of the rheological properties of reconstituted mucilage from four cactus pear cultivars. From the study, the authors noted the existence of an inverse relationship between temperature and the viscosity of the mucilage. The viscosity was measured at different temperatures from 20 to 40°C and it was observed that the viscosity of the mucilage decreased as the temperature increased. The researchers further observed an inverse relationship between the steady-shear viscosity and ionic strength of the mucilage, where one parameter increased as the other decreased. Trivalent ions were found to have the highest effects on the viscosity of the mucilage as compared to divalent and monovalent ions. A relationship between viscosity and pH was also observed with the viscosity increasing sharply as the solution moved from an acidic to a basic region. With regards to the effect of the year of harvest on the viscosity of the various cultivars using two methods, significant ($p < 0.001$) differences were only observed in the analysis which was done using a viscometer and not through the line-spread method (Table 11). Using a viscometer, the 2018 harvest (22.98 cP) contained less viscous mucilage as compared to 2019 (186.30 cP). One explanation for this may be the fact that the average environmental temperatures which were reported for 2018 (25.66°C) were higher than those reported for 2019 (22.98°C). Du Toit *et al.* (2020) reported lower mucilage viscosity in mucilage that was extracted from cladodes that were harvested in warmer months as compared to those that were harvested in colder months of the year. This also explains why the likes of Meyers, Messina, Fresno and R1260 recorded some of the highest viscosities in the analysis of viscosity using both the viscometer and line-spread method, when investigating the effect of cultivar on this attribute (Table 7). This in turn proves the observation that was made by Du Toit *et al.* (2017), in that an increase in temperature results in a decrease in viscosity. From an eating quality perspective, nopalitos with low mucilage are the best, as such, the nopalitos which were harvested in 2018 were the best in this aspect. When looking to use the mucilage in various product applications, one would say that the cultivars of the 2019 harvest would be the best as they would provide maximum viscosity at the least concentration.

3.4.4.3. The effect of year of harvest on the physico-chemical and chemical attributes of nopalitos

Table 12 shows the means of the physico-chemical and chemical attributes data of the 20 nopalito cultivars over two years.

Table 12: Analysis of variance (ANOVA) for the influence of year of harvest on the physico-chemical and chemical attributes of nopalitos

	2018	2019	Sign level
pH	$4.04^a \pm 0.15$	$4.27^b \pm 0.22$	$p < 0.001$
Moisture content (%)	86.23 ± 11.86	86.92 ± 18.24	$p = 0.730$
% Solids	13.77 ± 11.86	13.08 ± 18.24	$p = 0.730$
%Titratable acidity expressed as citric acid	$0.47^b \pm 0.11$	$0.26^a \pm 0.10$	$p < 0.001$

Sugar (TSS °Brix)	6.20 ± 1.13	5.94 ± 0.94	p = 0.055

Means with different superscripts in the same row differ significantly

E. pH

Because nopalitos operate on a CAM metabolism, their acidity levels are prone to vary throughout the day. It is with this kind of metabolism, that the concentration of titratable acid increases in the green tissue of the plants in the evening, while it decreases in the photosynthetic tissue during the day (Ranson & Thomas, 1960; Milburn *et al.,* 1968). The fluctuations in the acid content may be caused by the changes in the rate of photosynthesis or other pathways of metabolism that may replace the CAM metabolism in plants (Black, 1973). High concentrations of acid in cladodes have been reported to cause diarrhoea in grazing cattle and sheep (Yochai & Sigmund, 1975). As such, establishing a uniform time to harvest nopalitos is important to ensure that the acidity can be utilised for preservation purposes as well as to ensure that the vegetables are largely accepted by consumers (Berger *et al.*, 2013). The timing for which nopalitos can be harvested is an important factor, seemingly because harvesting seasons vary according to the agroclimatic conditions, the variety of cultivars being harvested as well as whether coerced blooming was utilised as a production technique (Berger *et al.,* 2013). Table 12 shows the effect of the year of harvest on the pH of the nopalitos, and from this, one can deduce that 2019 (4.27) had a significantly ($p < 0.001$) higher influence as compared to 2018 (4.07). This, in turn, implies that the 2018 harvest produced nopalitos which were more acidic and therefore, less desirable than those produced in 2019.

Albeit, the aforementioned protocol of harvesting at the same time in the two years was implemented in this study, one can deduce that other factors such as the agroclimatic conditions played a role in the differences in the pH of the nopalitos. One such factor that plays a role in the differences in pH, is temperature. It has been reported that the increase in temperature results in a decrease in pH (Du Toit *et al.,* 2020). Du Toit *et al.* (2019) examined the pH of mucilage from various cladode cultivars over a period of months and they observed that the pH of the samples increased in colder weather conditions (winter) as compared to warm weather conditions (summer). In February of that year, the average pH of the samples was 3.98, while it increased to 4.36 in August. Taking this into consideration, one may deduce that the observation is proven by the results of this study since 2019 reported higher pH levels as compared to 2018, seemingly because the former recorded lower temperatures on average.

F. *Titratable acidity*

Organic acids in foods such as acetic acid, citric acid and malic acid are known to play a significant role in the flavour, colour, and microbial stability, as well as the shelf life of food. The titratable acidity readings in these foods are expressed as a percentage of the predominant acid (Tyl & Sadler, 2017). In this study, citric acid was the predominant acid. This acid was found to be critical to the changes that take place in the cell-sap titratable acidity and vacuolar pH in early studies of CAM metabolism (Wolf, 1960). Guthrie (1934) detected a five-fold increase in citric acid levels in a CAM plant (*Bryophyllwn ealyeinum*). This increase was observed during the night and accounted for 25% of the change in total acidity in the plant. This observation supports Yochai and Sigmund's (1975) findings of a build-up of acids in cladodes of the *Opuntia* species at night and a decrease in acidity during the day. Furthermore, changes in the titratable acidity measurements of these cladodes showed CAM to dominate the photosynthetic metabolism.

In this study, significant ($p < 0.001$) differences were observed in the titratable acidity of the various nopalito cultivars between 2018 (0.47% expressed as citric acid) and 2019 (0.26% expressed as citric acid) (Table 12). The differences in this attribute in the nopalito cultivars may be a result of the time in which the nopalitos were harvested, the age, management as well as the area in which the nopalitos were produced (López-Palacios *et al.*, 2010; Meraz-Maldonado *et al.*, 2012). Yochai and Sigmund (1975) ascribed the rate of the changes in the titratable acidity to ecological conditions, wherein, cladodes that were in the shade, for example, showed to have a decreased rate of photodeacidification, while darkness stopped the process completely. In the case of consumption, one can deduce that 2019 produced better nopalitos than 2018 as the acidity was lower than the latter. Nopalitos that are high in acidity are of less desirable edible quality as they are too sour. A contributing factor to the increased acidity levels that were observed in the nopalitos harvested in 2018, may be the average temperatures of that year, which were generally higher than those that were recorded in 2019. Yochai and Sigmund (1975) also reported cladodes to have elevated acidity levels in the morning following a night of low temperatures.

3.4.5. The effect of cultivar and the year of harvest interaction on the various nopalito attributes

The evaluation of the effect of cultivar and the year of harvest interaction on the nopalitos is of the utmost importance as it provides insight into formulating the best planting and harvesting practices, to ensure maximum quality yield in every season. Tables 13, 14, 15 and 16 show the interaction between cultivar and the year of harvest and how this interaction affected the various properties of the nopalitos. From these tables, no significant differences were observed in the weight ($p = 0.180$), length ($p = 0.292$), width ($p = 0.071$), diameter ($p = 0.100$), pH (p = 0.072), compressibility (p = 0.011), firmness (p = 0.420), moisture content (p = 0.999), %solids (p = 0.999), and %waste (p = 0.064). The interaction between cultivar and the year of harvest showed significant differences and effects in the following attributes: the pulp weighed before the centrifugation process (g), extracted mucilage (g), %yield of mucilage, the waste obtained (g), the viscosity of the mucilage using a viscometer (cP), % titratable acidity expressed as citric acid, the viscosity of mucilage using the line-spread method (cm), sugar content (TSS °Brix) as well as the colour coordinates which will all be discussed below.

3.4.5.1. The effect of cultivar and the year of harvest interaction on the morphological and physiological attributes of nopalitos

Tables 13 and 14 show the means of the morphological characteristics, the yield of the pulp as well as mucilage data from the 20 nopalito cultivars and how these attributes were affected by the cultivar and the year of harvest interaction.

Table 13: Analysis of variance (ANOVA) for the effect of cultivar and the year harvest interaction on the morphological attributes of nopalitos

Cultivar	Year	weight (g)	length (cm)	width(cm)	diameter (cm)
Gymno Carpo	2018	65.36 ± 18.30	15.73 ±1.81	8.78 ± 1.47	0.35 ± 0.08
	2019	72.61 ± 27.71	16.33 ± 2.58	9.22 ± 1.77	0.50 ± 0.15
R1251	2018	66.79 ± 20.72	17.17 ± 3.14	8.27 ± 1.32	0.48 ± 0.13
	2019	62.44 ± 11.42	16.67 ± 1.37	8.28 ± 0.72	0.40 ± 0.06
Meyers	2018	71.47 ± 11.43	17.20 ± 1.09	8.90 ± 0.72	0.53 ± 0.12
	2019	82.84 ± 28.08	17.83 ± 2.48	10.25 ± 2.14	0.42 ± 0.10
Skinners Court	2018	66.67 ± 13.59	17.90 ± 1.62	6.58 ± 0.51	0.53 ± 0.16
	2019	73.81 ± 35.61	16.78 ± 2.61	8.08 ± 2.22	0.43 ± 0.12
Algerian	2018	102.44 ± 20.87	19.03 ± 0.62	10.70 ± 0.76	0.47 ± 0.10
	2019	65.63 ± 17.38	16.33 ± 2.66	8.47 ± 0.66	0.53 ± 0.05
Ofer	2018	53.63 ± 15.26	15.45 ± 1.65	8.08 ± 1.13	0.48 ± 0.15
	2019	66.51 ± 18.23	16.83 ± 1.83	8.78 ± 1.18	0.40 ± 0.01
Turpin	2018	73.92 ± 31.28	17.32 ± 1.84	8.67 ± 2.29	0.53 ± 0.24
	2019	58.97 ± 18.22	15.67 ± 2.25	8.85 ± 1.43	0.43 ± 0.05
Messina	2018	71.91 ± 17.63	16.05 ± 1.75	8.42 ± 1.20	0.57 ± 0.23
	2019	85.55 ± 53.66	16.67 ± 3.98	7.95 ± 1.91	0.48 ± 0.10
Santa Rossa	2018	78.14 ± 3.32	18.10 ± 0.53	9.05 ± 0.45	0.35 ± 0.05
	2019	141.08 ± 53.11	21.17 ± 2.64	11.40 ± 2.23	0.48 ± 0.17
Berg X Mexican	2018	70.40 ± 20.29	15.65 ± 2.12	9.00 ± 1.56	0.50 ± 0.20
	2019	64.56 ± 26.95	16.33 ± 2.58	8.62 ± 1.33	0.42 ± 0.10
R1260	2018	64.05 ± 18.31	16.40 ± 2.69	7.92 ± 1.06	0.43 ± 0.15

	2019	87.29 ± 55.36	18.17 ± 4.92	9.39 ± 2.84	0.48 ± 0.21
Fresno	2018	171.67 ± 43.24	18.62 ± 1.47	9.72 ± 0.92	0.86 ± 0.08
	2019	139.53 ± 33.11	20.17 ± 3.66	11.23 ± 1.78	0.91 ± 0.26
Fusicaulis	2018	144.73 ± 33.99	17.40 ± 3.14	8.52 ± 1.89	1.05 ± 0.10
	2019	154.34 ± 67.85	17.50 ± 2.43	9.75 ± 1.47	0.88 ± 0.18
Malta	2018	74.93 ± 19.41	17.97 ± 2.33	9.13 ± 1.00	0.48 ± 0.10
	2019	56.52 ± 14.75	15.67 ± 1.37	8.77 ± 1.21	0.45 ± 0.05
Morado	2018	71.63 ± 26.45	17.28 ± 3.22	9.22 ± 2.06	0.43 ± 0.16
	2019	56.31 ± 24.92	15.17 ± 2.86	8.28 ± 1.48	0.42 ± 0.08
Nudosa	2018	153.98 ± 42.39	19.07 ± 2.05	11.31 ± 0.81	0.77 ± 0.29
	2019	152.28 ± 69.42	19.67 ± 3.01	11.20 ± 1.93	0.91 ± 0.27
Robusta	2018	147.83 ± 46.39	17.03 ± 2.38	11.11 ± 1.14	0.80 ± 0.26
	2019	154.23 ± 47.21	17.17 ± 2.48	11.19 ± 0.54	0.97 ± 0.27
Zastron	2018	66.91 ± 21.05	16.33 ± 2.14	7.78 ± 1.13	0.45 ± 0.16
	2019	68.40 ± 14.24	17.33 ± 1.63	7.85 ± 0.68	0.58 ± 0.19
Rossa	2018	69.87 ± 13.77	16.92 ± 1.77	8.68 ± 1.25	0.38 ± 0.08
	2019	72.80 ± 29.51	16.17 ± 1.47	8.63 ± 0.73	0.53 ± 0.14
Sicilian Indian Fig	2018	84.41 ± 16.75	17.48 ± 1.59	8.63 ± 0.79	0.62 ± 0.20
	2019	71.81 ± 26.49	16.33 ± 2.66	8.90 ± 1.38	0.52 ± 0.12
Average		88.96	17.20	9.09	0.55
Sign. level		p = 0.180	p = 0.292	p = 0.071	p = 0.100

Means with different superscripts in the same column differ significantly

Table 14: Analysis of variance (ANOVA) for the effect of cultivar and year harvest interaction on the yield of pulp and mucilage data from the 20 nopalito cultivars

Cultivar	Year	Pulp (before centrifuge) (g)	Extracted mucilage (g)	Waste (g)	%yield	%waste
Gymno Carpo	2018	35.91^{ab} ± 16.84	8.23^{abc} ± 4.99	18.30^{a} ± 5.42	12.75^{abcde} ± 6.74	29.40 ± 10.73
	2019	56.31^{abcd} ± 24.38	14.28^{abc} ± 7.40	41.71^{abcde} ± 16.61	19.13^{abcdefgh} ± 4.39	59.67 ± 13.59
R1251	2018	22.76^{a} ± 9.06	10.33^{abc} ± 10.92	20.98^{ab} ± 9.36	13.23^{abcdef} ± 13.14	31.96 ± 13.56
	2019	42.81^{abc} ± 12.44	15.17^{abc} ± 8.03	25.64^{abc} ± 6.13	23.17^{abcdefgh} ± 8.40	41.32 ± 7.35
Meyers	2018	29.34^{a} ± 7.16	10.40^{abc} ± 6.25	18.22^{a} ± 3.03	13.81^{abcdef} ± 6.60	25.89 ± 4.98
	2019	67.64^{abcdefg} ± 20.27	34.02^{abcdef} ± 4.14	34.17^{abcd} ± 10.40	34.04^{defghi} ± 5.48	43.06 ± 8.45
Skinners Court	2018	28.57^{a} ± 15.91	1.89^{a} ± 1.97	22.48^{ab} ± 4.93	2.73^{a} ± 2.97	34.26 ± 6.74
	2019	53.28^{abc} ± 37.01	19.43^{abcd} ± 25.45	33.58^{abcd} ± 14.56	19.42^{abcdefgh} ± 21.32	47.74 ± 10.68
Algerian	2018	61.93^{abcde} ± 29.61	22.04^{abcd} ± 6.79	32.03^{abcd} ± 12.06	21.53^{abcdefgh} ± 5.18	31.34 ± 9.45
	2019	49.48^{abc} ± 18.32	13.84^{abc} ± 10.15	34.11^{abcd} ± 8.45	19.12^{abcdefgh} ± 11.63	52.35 ± 3.77
Ofer	2018	22.09^{a} ± 11.33	2.71^{a} ± 2.18	18.97^{ab} ± 5.76	4.98^{a} ± 4.22	36.04 ± 10.07
	2019	49.10^{abc} ± 19.34	13.02^{abc} ± 10.42	34.52^{abcd} ± 9.33	17.65^{abcdefg} ± 9.15	52.21 ± 6.36
Turpin	2018	30.01^{a} ± 11.60	11.86^{abc} ± 8.64	21.28^{ab} ± 15.76	14.66^{abcdef} ± 10.62	29.06 ± 23.28
	2019	41.63^{abc} ± 18.77	10.70^{abc} ± 9.00	28.53^{abc} ± 13.57	17.13^{abcdefg} ± 10.41	47.14 ± 9.45
Messina	2018	31.71^{a} ± 19.59	8.61^{abc} ± 10.01	24.39^{abc} ± 11.49	9.85^{abc} ± 11.13	32.69 ± 8.72
	2019	62.88^{abcde} ± 46.58	28.77^{abcde} ± 27.72	32.12^{abcd} ± 17.36	23.93^{abcdefgh} ± 13.93	42.86 ± 11.58
Santa Rossa	2018	31.65^{a} ± 1.00	9.31^{abc} ± 1.77	17.83^{a} ± 5.49	11.93^{abcd} ± 2.25	22.96 ± 7.52
	2019	124.64^{efg} ± 54.48	65.26^{efg} ± 31.37	45.49^{abcde} ± 15.23	42.55^{hi} ± 18.16	34.85 ± 10.12
Berg X Mexican	2018	30.24^{a} ± 7.80	12.87^{abc} ± 4.34	16.54^{a} ± 2.37	18.14^{abcdefg} ± 2.80	24.51 ± 4.94
	2019	48.03^{abc} ± 22.06	16.78^{abcd} ± 11.62	29.98^{abcd} ± 9.37	23.65^{abcdefgh} ± 7.85	47.89 ± 4.44
R1260	2018	29.53^{a} ± 5.18	12.26^{abc} ± 3.37	15.72^{a} ± 1.58	19.20^{abcdefgh} ± 1.32	26.34 ± 8.31

	2019	$70.01^{abcdefg}$ ± 47.19	31.59^{abcde} ± 25.35	38.04^{abcd} ± 18.08	33.52^{defghi} ± 16.48	42.42 ± 20.67
Fresno	2018	64.19^{abcdef} ± 25.89	25.08^{abcd} ± 13.77	27.37^{abc} ± 18.52	12.52^{abcd} ± 4.79	13.36 ± 6.39
	2019	$78.97^{abcdefg}$ ± 22.30	43.38^{bcdef} ± 24.79	70.06^{e} ± 31.45	40.32^{ghi} ± 11.20	38.83 ± 11.36
Fusicaulis	2018	128.40^{fg} ± 34.58	84.83^{g} ± 26.39	42.53^{abcde} ± 8.73	52.05^{i} ± 13.47	30.77 ± 9.96
	2019	129.61^{g} ± 58.72	54.43^{defg} ± 34.29	59.53^{de} ± 21.00	$32.49^{cdefghi}$ ± 8.17	45.38 ± 3.14
Malta	2018	40.13^{ab} ± 21.49	11.61^{abc} ± 9.52	26.98^{abc} ± 12.47	13.96^{abcdef} ± 9.77	34.85 ± 8.19
	2019	38.57^{ab} ± 10.80	7.37^{ab} ± 4.57	29.86^{abcd} ± 5.94	12.40^{abcd} ± 6.45	53.53 ± 4.86
Morado	2018	29.86^{a} ± 7.55	9.24^{abc} ± 5.18	19.27^{ab} ± 3.80	12.51^{abcd} ± 4.02	28.57 ± 6.04
	2019	41.45^{abc} ± 18.45	9.83^{abc} ± 5.99	28.22^{abc} ± 9.65	16.58^{abcdef} ± 7.83	52.06 ± 6.47
Nudosa	2018	105.06^{cdefg} ± 34.76	47.16^{cdefg} ± 19.30	48.51^{bcde} ± 18.82	$30.30^{bcdefghi}$ ± 10.51	30.84 ± 8.82
	2019	120.66^{defg} ± 63.29	70.98^{fg} ± 61.16	52.98^{cde} ± 17.33	36.28^{fghi} ± 14.44	44.53 ± 7.94
Robusta	2018	$70.78^{abcdefg}$ ± 62.39	21.80^{abcd} ± 4.59	28.97^{abc} ± 25.41	$22.62^{abcdefgh}$ ± 13.64	13.16 ± 5.55
	2019	100.21^{bcdefg} ± 28.67	33.59^{abcdef} ± 11.93	52.77^{cde} ± 19.18	36.05^{efghi} ± 16.55	45.87 ± 18.69
Zastron	2018	25.37^{a} ± 8.04	6.69^{ab} ± 5.55	24.08^{abc} ± 9.54	8.64^{ab} ± 6.09	35.81 ± 8.27
	2019	55.76^{abc} ± 13.37	14.06^{abc} ± 6.13	35.59^{abcd} ± 7.10	$20.68^{abcdefgh}$ ± 7.14	52.40 ± 5.87
Rossa	2018	27.31^{a} ± 8.58	11.33^{abc} ± 5.94	16.56^{a} ± 4.98	15.03^{abcdef} ± 7.70	23.66 ± 5.60
	2019	53.89^{abc} ± 26.62	15.10^{abc} ± 16.02	38.07^{abcd} ± 13.55	$18.19^{abcdefg}$ ± 13.57	53.50 ± 9.98
Sicilian Indian Fig	2018	36.35^{ab} ± 6.47	15.37^{abc} ± 6.87	20.74^{ab} ± 3.22	$18.24^{abcdefg}$ ± 7.80	25.19 ± 4.86
	2019	56.92^{abcd} ± 21.25	15.23^{abc} ± 12.15	39.65^{abcd} ± 12.34	$18.63^{abcdefg}$ ± 11.03	57.81 ± 16.28
Average		55.58	21.76	32.98	20.84	37.90
Sign. level		$p = 0.046$	$p < 0.001$	$p = 0.036$	$p < 0.001$	$p = 0.064$

Means with different superscripts in the same column differ significantly

G. Weight

The weight of the various nopalito cultivars ranged between 53.63 g (Ofer) and 171.67 g (Fresno) between the two years (2018 and 2019), with an average of 88.96 g (Table 13). No significant differences were observed in this investigation. One can recall that when observing the effect of cultivar on the various nopalito attributes, Ofer and Fresno, seemingly weighed the least and the heaviest, respectively (Table 6). Furthermore, the same cultivars which were observed to be the heaviest in the investigation of the effect of cultivar on the various nopalito attributes were found to be the same in the investigation of the effect of cultivar and the year of harvest on the various attributes. These heavy nopalito cultivars are Fresno (171.67 g in 2018 and 139.53 g in 2019), Fusicaulis (144.73 g in 2018 and 154.34 g in 2019), Nudosa (153.98 g in 2018 and 152.28 g in 2019) and Robusta (147.83 g in 2018 and 154.23 g in 2019).

H. Size

The length of the nopalitos ranged between 15.17 cm (Morado) and 21.17 cm (Santa Rossa), with an average of 17.20 cm between the two years (Table 13). No significant differences were observed in this investigation. Berg X Mexican (15.65 cm in 2018 and 16.33 cm in 2019) and Gymno Carpo (15.73 cm in 2018 and 16.33 cm in 2019) which scored the lowest in the effect of cultivar on the various nopalito attributes (Table 6), also recorded some of the lowest measurements under this investigation. The same trend was observed with Santa Rossa (18.10 cm in 2018 and 21.17 cm in 2019), which served as the longest-measured cultivar in both investigations (Table 6).

The widest nopalito cultivar which was measured was Santa Rossa (11.40 cm) in 2019 and Nudosa (11.31 cm) in the 2018 season. The narrowest cultivar measured was Skinners Court (6.58 cm) which was in 2018, while it was Zastron (7.85 cm) in 2019. No significant differences were observed in the width of the cultivars over the two years, with the majority ranging between 8.08 cm (Ofer) and 9.75 cm (Fusicaulis) (Table 13).

Generally, the widest cultivars that were recorded over the two years were Santa Rossa (11.40 cm in 2019), Nudosa (11.31 cm in 2018 and 11.20 cm in 2019), Fresno (11.23 cm in 2019), Robusta (11.11 cm in 2018 and 11.19 cm in 2019), Algerian (10.70 cm in 2018) and Meyers (10.25 cm in 2019). On the other side of the spectrum, the narrowest cultivars over the two years were Skinners Court (6.58 cm in 2018), Messina (7.95 cm in 2019), R1260 (7.92 cm in 2018) and Zastron (7.78 cm in 2018 and 7.85 cm in 2019).

The diameter of the nopalitos ranged between 0.35 cm (Gymno Carpo and Santa Rossa) and 1.05 cm (Fusicaulis), with an average of 0.55 cm (Table 13). No significant differences were observed in this investigation with the majority of the cultivars ranging between 0.40 cm and 0.57 cm. Generally, the greatest diameters that were recorded among the cultivars over the two years were Fusicaulis (1.05 cm in 2018 and 0.88 cm in 2019), Robusta (0.97 cm in 2019), Nudosa (0.77 cm in 2018 and 0.91 cm in 2019), Fresno (0.86 cm in 2018 and 0.91 cm in 2019) and Sicilian Indian Fig (0.62 cm in 2018). On the other side of the spectrum, the smallest diameters that were recorded over the two years were observed in Gymno Carpo (0.35 cm in 2018), Santa Rossa (0.35 cm in 2018) and Rossa (0.38 cm in 2018). The effect of cultivar on the various nopalito properties revealed a corresponding pattern in that, Gymno Carpo and Santa Rossa recorded the lowest diameter. At the same time, Fusicaulis measured the highest diameter out of the 20 cultivars (Table 6).

I. Extracted mucilage yield and waste

The amount of mucilage that was extracted over the two years ranged between 1.89 g (Skinners Court) and 84.83 g (Fusicaulis), with several significant ($p < 0.001$) differences being observed (Table 14). Generally, the highest amount of mucilage which was extracted was from the cultivars that were harvested in 2019 as compared to 2018. This further supports the observation that was made in the investigation of the effect of the year of harvest on the various attributes, which showed 2019 to have had a significantly ($p = 0.004$) higher amount of extracted mucilage compared to 2018 (Table 11). Noticeable amounts of extracted mucilage were observed in Fusicaulis (84.83 g in 2018 and 43.38 g in 2019), Nudosa (47.16 g in 2018 and 70.98 g in 2019), Santa Rossa (65.26 g in 2019), Fusicaulis (54.43 g in 2019), Meyers (34.02 g in 2019), Robusta (33.59 g in 2019), R1260 (31. 59 g in 2019) and Messina (28.77 g in 2019). The greatest differences that were observed in the amount of extracted mucilage between the two years were found in some cultivars. For example, some noticeable differences were observed in Santa Rossa whose mucilage increased from 9.31 g in 2018 to a staggering 65.26 g in 2019, which was a difference of 55.95 g. Other cultivars included Fusicaulis which yielded a difference of 30.40 g between 2018 (84.83 g) and 2019 (54.43 g); Nudosa which yielded a difference of 23.82 g between 2018 (47.16 g) and 2019 (70.98 g); Meyers yielded a difference of 23.62 g between the two years as it yielded a mucilage content of 10.40 g in 2018, while it yielded a mucilage content of 34.02 g in 2019; lastly, the biggest yield difference was observed in Messina whose mucilage which was extracted in 2018 amounted to 8.61 g, while it amounted to 28.77 g in 2019. These vast differences between the two years may be ascribed to the moisture content found in these cultivars, as it directly affects the mucilage content of these cladodes. The nopalitos which were harvested in 2019, recorded higher contents of moisture as compared to 2018 (Table 12). As such, it is not a surprise that the extracted mucilage content was higher in 2019 as compared to 2018.

Significant ($p < 0.001$) differences were observed among the cultivars in terms of the %yield of the extracted mucilage, which ranged between 2.73% (Skinners Court) and 52.05% (Fusicaulis), with an average of 20.84% (Table 14). The %yield that was obtained correlates with the amount of extracted mucilage that was recorded, in that the 2019 harvest resulted in a higher yield. Some of these high mucilage yields were observed in Fusicaulis (52.05% in 2018 and 32.49% in 2019), Santa Rossa (42.55% in 2019), Fresno (40.32% in 2019), Nudosa (36.28% in 2019), Robusta (36.05% in 2019), Meyers (34.04% in 2019) and R1260 (33.52% in 2019). The least amount of mucilage yield was observed in Skinners Court (2.73%), Ofer (4.98%), Zastron (8.64%) and Messina (9.85%). All these values were observed in 2018. The vast differences among the %yields in mucilage prove that the cultivars did have a significant influence on this attribute, as shown in Table 5.

Significant (p = 0.046) differences were observed among the cultivars in terms of the pulp that was obtained before centrifugation (Table 14). The most pulp that was obtained from the nopalitos before the centrifugation process was observed in 2019 with Fusicaulis yielding the highest pulp in both years (128.40 g in 2018 and 129.61 g in 2019). On the other side of the spectrum, the least amount of pulp recorded was from Ofer (22.09 g in 2018), R1251 (22.76 g in 2018), Zastron (25.37 g in 2018), Rossa (27.31 g in 2018), R1260 (29.53 g in 2018), Skinners Court (28.57 g in 2018), Meyers (29.34 g in 2018), Morado (29.86 g in 2018), Turpin (30.01 g in 2018), Berg X Mexican (30.24 g in 2018), Santa Rossa (31.65 g in 2018) and Messina (31.71 g in 2018). The most remarkable differences which were observed between the cultivars' initial weights and their macerated pulps obtained before centrifugation were in Algerian (original weight of 102.44 g), Nudosa (original weight of 153.98 g) and Robusta (original weight of 147.83 g) (Table 6). These cultivars lost the highest amount of weight in the form of steam during the cooking process, which resulted in a final pulp weight of 61.93 g, 105.06 g and 70.78 g, respectively. It must be noted that the cooking process and therefore, the loss of steam did not have an effect on the mucilage obtained after the centrifugation process as Nudosa and Robusta still yielded some of the highest amounts of mucilage despite the notable weight differences (Table 14).

The waste that was recorded among the nopalito cultivars in the two years showed some significant (p = 0.36) differences (Table 14). However, no significant (p = 0.64) differences were observed in the % Waste obtained (Table 14). The waste ranged between 15.72 g/26.34% (R1260 in 2018) and 70.06 g/38.83% (Fresno in 2019), with an average of 32.98 g/37.90%. The least amount of waste was observed in R1260 (15.72 g/26.34%), Berg X Mexican (16.54 g/24.51% in 2018), Rossa (16.56 g/23.66% in 2018), Santa Rossa (17.83 g/22.96% in 2018), Gymno Carpo (18.30 g/29.40% in 2018) and Meyers (18.22 g/25.89% in 2018). On the other side of the spectrum, Fresno (70.06 g/26.34% in 2019), Fusicaulis (42.53 g/30.77% in 2018 and 59.53 g/45.38% in 2019), Nudosa (48.51 g/30.84%52.98 g/44.53% in 2019), Robusta (52.77 g/45.87% in 2019), Santa Rossa (45.49 g/34.85%) and Gymno Carpo (41.71 g/59.67% in 2019). The results observed suggest that the most amount of waste was obtained in 2019 as compared to 2018, which coincides with the observation that was made of this parameter under the investigation of the effect of the year of harvest on waste generated (Table 10). Furthermore, some of the cultivars which were recorded to have had the least and most waste generated, respectively, in this instance, were also observed to have done so in the investigation of the effect of the cultivar on this attribute (Table 7). For example, Fusicaulis, Nudosa and Fresno recorded some of the highest amounts of waste in Table 6, while Berg X Mexican, R1251, Morado and Turpin yielded the lowest amount of waste.

3.4.5.2. The effect of cultivar and the year of harvest interaction on the physical attributes of nopalitos

J. Colour

In Table 15, the colour attributes of the 20 different nopalitos cultivars are observed and how they were affected by the cultivar and the year of harvest interaction.

Table 15: Analysis of variance (ANOVA) for the effect of the interaction between cultivar and the year of harvest on the colour properties of nopalitos

Cultivar	**Year**	**L**	**A**	**b**	**Chroma (Saturation Index)**	**Hue Angle**
Gymno Carpo	2018	56.28^{g} ± 1.23	-21.23^{a} ± 1.05	38.27^{i} ± 2.50	43.77^{h} ± 2.64	-60.95^{a} ± 0.83
	2019	50.48^{bcdefg} ± 1.29	-19.09^{abcd} ± 1.70	30.34^{bcdefghi} ± 3.64	35.85^{cdefgh} ± 3.95	-57.74^{abcdef} ± 1.20
R1251	2018	53.12^{defg} ± 1.97	-20.31^{abc} ± 0.67	33.90^{defghi} ± 3.26	39.53^{efgh} ± 3.09	-58.96^{abcd} ± 1.84
	2019	51.58^{cdefg} ± 2.71	-18.96^{abcd} ± 0.89	30.35^{bcdefghi} ± 3.05	35.80^{cdefgh} ± 2.97	-57.89^{abcdef} ± 1.83
Meyers	2018	54.57^{fg} ± 2.79	-21.39^{a} ± 0.51	37.46^{hi} ± 2.76	43.15^{h} ± 2.54	-60.20^{ab} ± 1.57
	2019	50.21^{abcdefg} ± 3.09	-17.66^{abcde} ± 6.37	29.66^{bcdefghi} ± 8.76	34.62^{bcdefgh} ± 10.43	-58.05^{abcdef} ± 2.41
Skinners Court	2018	50.26^{bcdefg} ± 1.80	-19.16^{abcd} ± 1.64	31.48^{bcdefghi} ± 4.12	36.87^{cdefgh} ± 4.26	-58.54^{abcd} ± 1.97
	2019	48.27^{abcdef} ± 2.65	-16.71^{abcde} ± 3.69	26.81^{abcdefghi} ± 8.12	31.65^{bcdefgh} ± 8.67	-57.29^{abcdef} ± 3.99
Algerian	2018	53.17^{defg} ± 3.02	-19.91^{abc} ± 1.01	33.47^{defghi} ± 3.97	38.96^{defgh} ± 3.89	-59.11^{abc} ± 1.93
	2019	43.84^{ab} ± 3.25	-11.90^{de} ± 3.07	15.66^{a} ± 5.48	19.69^{ab} ± 6.22	-52.23^{efg} ± 2.38
Ofer	2018	53.25^{defg} ± 2.02	-19.83^{abc} ± 1.57	33.86^{defghi} ± 3.87	39.24^{defgh} ± 4.10	-59.55^{abc} ± 1.20
	2019	46.99^{abcde} ± 3.44	-14.38^{abcde} ± 4.20	20.55^{abcd} ± 7.55	25.10^{abcde} ± 8.57	-54.43^{bcdefg} ± 2.43
Turpin	2018	53.83^{efg} ± 2.48	-20.30^{abc} ± 1.22	34.68^{efghi} ± 5.00	40.22^{efgh} ± 4.82	-59.42^{abc} ± 2.49
	2019	51.65^{cdefg} ± 3.37	-18.19^{abcd} ± 1.17	30.07^{bcdefghi} ± 1.66	35.14^{cdefgh} ± 1.97	-58.84^{abcd} ± 0.77
Messina	2018	52.12^{cdefg} ± 1.52	-19.35^{abcd} ± 1.76	33.63^{defghi} ± 4.07	38.81^{defgh} ± 4.34	-60.01^{ab} ± 1.36
	2019	50.73^{bcdefg} ± 3.57	-18.99^{abcd} ± 1.38	31.50^{bcdefghi} ± 3.82	36.80^{cdefgh} ± 3.93	-58.79^{abcd} ± 1.67
Santa Rossa	2018	54.06^{efg} ± 1.97	-19.62^{abc} ± 1.08	34.50^{efghi} ± 3.80	38.86^{defgh} ± 3.74	-59.40^{abc} ± 2.00
	2019	50.48^{bcdefg} ± 4.09	-17.02^{abcde} ± 4.80	28.30^{abcdefghi} ± 4.98	33.10^{bcdefgh} ± 6.49	-57.80^{abcdef} ± 1.24
Berg X Mexican	2018	53.38^{defg} ± 0.78	-21.27^{a} ± 1.08	35.90^{ghi} ± 3.13	41.73^{gh} ± 3.21	-59.29^{abc} ± 1.11
	2019	43.54^{ab} ± 5.49	-13.05^{cde} ± 5.58	20.37^{abcd} ± 11.72	24.31^{abcd} ± 12.71	-55.68^{abcdef} ± 7.08
R1260	2018	54.53^{fg} ± 3.25	-20.68^{ab} ± 1.17	35.48^{fghi} ± 4.84	41.09^{fgh} ± 4.70	-59.58^{abc} ± 2.23

	2019	48.44^{abcdef} ± 4.09	-18.71^{abcd} ± 1.66	30.19^{bcdefghi} ± 3.11	35.52^{cdefgh} ± 3.44	-58.17^{abcde} ± 1.23
Fresno	2018	45.05^{abc} ± 3.03	-14.37^{abcde} ± 3.06	19.55^{abc} ± 4.79	24.28^{abcd} ± 5.62	-53.53^{cdefg} ± 2.30
	2019	47.57^{abcdef} ± 4.56	-14.22^{abcde} ± 5.71	21.75^{abcdef} ± 11.35	26.03^{abcdef} ± 12.61	-55^{abcdef}.30 ± 3.78
Fusicaulis	2018	42.93^{a} ± 1.66	-14.01^{abcde} ± 1.40	17.90^{ab} ± 1.63	22.74^{abc} ± 2.06	-51.97^{fg} ± 1.47
	2019	47.73^{abcdef} ± 3.32	-14.24^{abcde} ± 6.89	23.50^{abcdefg} ± 9.12	27.53^{abcdefg} ± 11.26	-60.22^{ab} ± 4.96
Malta	2018	54.10^{efg} ± 1.51	-20.61^{ab} ± 1.84	35.07^{fghi} ± 4.66	40.69^{fgh} ± 4.90	-59.43^{abc} ± 1.49
	2019	45.36^{abc} ± 3.35	-10.29^{e} ± 2.98	14.92^{a} ± 6.73	15.83^{a} ± 5.82	-48.55^{g} ± 2.93
Morado	2018	54.02^{efg} ± 2.15	-20.89^{ab} ± 1.04	35.39^{fghi} ± 3.19	41.11^{fgh} ± 3.15	-59.36^{abc} ± 1.61
	2019	45.63^{abc} ± 6.19	-15.70^{abcde} ± 4.66	20.96^{abcde} ± 8.90	26.62^{abcdefg} ± 8.58	-56.69^{abcdef} ± 3.05
Nudosa	2018	50.11^{abcdefg} ± 4.99	-17.68^{abcde} ± 5.66	27.53^{abcdefghi} ± 12.02	32.78^{bcdefgh} ± 13.11	-55.85^{abcdef} ± 4.40
	2019	47.18^{abcde} ± 1.61	-16.71^{abcde} ± 1.35	24.95^{abcdefghi} ± 2.48	30.04^{abcdefgh} ± 2.75	-56.14^{abcdef} ± 1.23
Robusta	2018	48.95^{abcdef} ± 1.91	-13.65^{bcde} ± 4.32	18.89^{abc} ± 8.22	23.34^{abc} ± 9.17	-52.9^{defg} ± 3.85
	2019	45.74^{abc} ± 4.95	-16.26^{abcde} ± 4.76	24.45^{abcdefgh} ± 7.95	29.40^{abcdefgh} ± 9.13	-56.31^{abcdef} ± 3.18
Zastron	2018	51.25^{cdefg} ± 2.04	-19.99^{abc} ± 1.23	32.07^{cdefghi} ± 3.40	37.80^{cdefgh} ± 3.50	-57.96^{abcdef} ± 1.49
	2019	48.20^{abcdef} ± 3.11	-15.44^{abcde} ± 4.86	24.93^{abcdefghi} ± 6.26	29.35^{abcdefgh} ± 7.80	-58.95^{abcd} ± 3.63
Rossa	2018	54.65^{fg} ± 2.23	-20.39^{abc} ± 1.18	35.37^{fghhi} ± 4.23	40.84^{fgh} ± 4.15	-59.90^{ab} ± 2.00
	2019	49.59^{abcdefg} ± 4.91	-16.02^{abcde} ± 5.04	26.77^{abcdefghi} ± 8.09	31.21^{bcdefgh} ± 9.46	-59.37^{abc} ± 2.50
Sicilian Indian Fig	2018	53.93^{efg} ± 4.10	-18.69^{abcd} ± 0.98	30.40^{bcdefghi} ± 2.64	35.70^{cdefgh} ± 2.72	-58.35^{abcd} ± 1.23
	2019	46.43^{abcd} ± 1.81	-16.10^{abcde} ± 4.87	25.40^{abcdefghi} ± 6.06	30.11^{abcdefgh} ± 7.60	-58.28^{abcde} ± 3.98
Average		50.08	-17.57	28.41	33.38	57.52
Sign. Level		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

Means with different superscripts in the same column differ significantly

All colour coordinates that were investigated in the various nopalitos showed significant ($p < 0.001$) differences (Table 15). The lightness (L*) of the nopalitos ranged between 42.93 (Fusicaulis) and 56.28 (Gymno Carpo), with an average of 50.08. Nopalitos which were harvested in 2018 were generally lighter than those harvested in 2019, with 18 cultivars out of the 20 recording higher values in 2018. This observation correlates with the observation that was made in the investigation of the effect of the year of harvest on this particular attribute (Table 11). The dull cultivars were Fusicaulis (42.93 in 2018), Algerian (43.54 in 2019), Morado (45.63 in 2019), Robusta (45.74 in 2019), Sicilian Indian Fig (46.43 in 2019) as well as Ofer (46.99 in 2019). On the other side of the spectrum, the lightest cultivars that were harvested are Gymno Carpo (56.28 in 2018), Rossa (54.65 in 2018), Meyers (54.57 in 2018), R1260 (54.53 in 2018), Malta (54.10 in 2018), Santa Rossa (54.06 in 2018), Sicilian Indian Fig (53.93 in 2018) and Turpin (53.83 in 2018). Sicilian Indian Fig is a good example of how attributes of a cultivar can change over the years as it was deemed as one of the dullest looking cultivars in 2019, yet it was observed as one of the lightest looking cultivars in 2018. No significant differences were observed between Gymno Carpo (50.48 in 2019), Skinners Court (50.26 in 2018), as well as Messina (50.73 in 2019).

The a* values of the various nopalito cultivars ranged between -21.39 (Meyers) and -10.29 (Malta) over the two years, with an average of -17.57. No significant differences were observed in the majority of the cultivars over the two years. The least green tones were observed in Malta (-10.29 in 2019), Algerian (-11.90 in 2019), Berg X Mexican (-13.05 in 2019 as well as Robusta (-13.65 in 2018), while the greenest tones were observed in Meyers (-21.39), Berg X Mexican (-21.27 in 2018), Gymno Carpo (-21.23 in 2018), Morado (-20.89 in 2018), R1260 (-20.68 in 2018), Malta (-20.61 in 2018), Rossa (-20.39 in 2018), as well as Turpin (-20.30 in 2018). Generally, the 2018 harvest produced nopalitos which were greener in tone as compared to 2019. This observation correlates with the one that was made under the investigation of the effect of the year of harvest on this attribute (Table 11). Another clear example of this observation was with the cultivar Berg X Mexican which had one of the least green tones in 2019 (-13.05), while it was observed to have possessed the greenest tones in 2018 (-21.68).

Yellow tones (b*) among the nopalitos ranged between 14.92 (Malta) and 38.27 (Gymno Carpo) over the two years, with an average of 28.41 (Table 15). The least yellow tones were observed in Malta (14.92 in 2019), Algerian (15.66 in 2019), Fusicaulis (17.90 in 2018), Robusta (18.89 in 2018) and Fresno (19.55 in 2018). The cultivars which showed the most yellow tones are Gymno Carpo (38.27 in 2018), Meyers (37.48 in 2018), Berg X Mexican (35.90 in 2018), R1260 (35.48 in 2018), Morado (35.37 in 2018). Malta (35.07 in 2018), Turpin (34.68 in 2018), Santa Rossa (34.50 in 2018), 1251 (33.90 in 2018), Ofer (33.86 in 2018), Messina (33.63 in 2018) as well as Algerian (33.47 in 2018). Yellow tones were mostly noticeable in the nopalitos that were harvested in 2018, with 17 out of the 20 cultivars having shown greater yellow tones in the 2018 harvest as compared to 2019. The biggest differences which were observed in the cultivars over the two years were Malta and Algerian. Malta (14.92) recorded the least noticeable yellow tones in 2019, yet the same cultivar was mentioned among the cultivars, which yielded the highest degrees of yellow tones in 2018 (35.07). The same was observed with Algerian which was recorded as the second least (15.66) cultivar to possess yellow tones in 2019, yet it was among some of the highest yellow tone-containing cultivars in 2018 (33.47). This proves the observation that was made in the investigation of the effect of the year of harvest on this attribute, whereby 2018 showed greater significant ($p < 0.001$) differences to 2019 (Table 11). Another interesting observation was that of the blue-green-looking Robusta (18.89 in 2018 and 24.45 in 2019). Because of its nature, one would expect it to have recorded the lowest yellow tones. However, it was higher than some of the cultivars in the study and was still in the same ranges as Fresno (19.55 in 2018 and 21.75 in 2019) and Fusicaulis (17.90 in 2018 and 23.50 in 2019) as observed under the investigation of the effect of cultivar on this particular attribute (Table 8).

The brightness values (Chroma) of the nopalito cultivars ranged between 15.83 (Malta) and 43.77 (Gymno Carpo) over the two years, with an average of 33.38 (Table 15). The brightest cultivars that were recorded are Gymno Carpo (43.77 in 2018), Meyers (43.15 in 2018), Berg X Mexican (41.73 in 2018), Morado (41.11 in 2018), R1260 (41.09 in 2018), Rossa (40.84 in 2018), Malta (40.69 in 2018), Turpin (40.22 in 2018), R1251 (39.53 in 2018), Ofer (39.24 in 2018), Algerian (38.96 in 2018), Santa Rossa (38.86 in 2018) and Messina (38.81 in 2018). The least bright cultivars were Malta (15.83 in 2019), Algerian (19.69 in 2019), Fusicaulis (22.74 in 2018) and Robusta (23.34 in 2018). No significant differences were observed between Zastron (37.80 in 2018), Skinners Court (36.87 in 2018), Messina (36.80 in 2019), Gymno Carpo (35.85 in 2019), Sicilian Indian Fig (35.70 in 2018), R1260 (35.52 in 2019) and Turpin (35.14 in 2019). Other non-significant differences were observed between Meyers (34.62 in 2019), Santa Rossa (33.10 in 2019), Nudosa (32.78 in 2018), Skinners Court (31.65 in 2019) as well as Rossa (31.21 in 2019). The brightest nopalitos were observed in 2018 as compared to 2019, with 17 out of the 20 cultivars being brighter in 2018. This proves the observation that was made in the investigation of the effect of the year of harvest on this particular attribute, whereby 2018 was significantly ($p < 0.001$) greater than 2019 (Table 11). The biggest differences that were observed in cultivars over the two years were Algerian and Malta. Malta which was the least bright in 2019 (15.83), turned out to be one of the brightest cultivars in 2018 (40.69). The same observation was made with Algerian, where it was the second least bright cultivar in 2019 (19.64), whereas it recorded a relatively high level of brightness in 2018 (38.96).

The hues (h°) of the various nopalito cultivars ranged between -48.55 (Malta) and -60.95 (Gymno Carpo), with an average of -57.52 (Table 15). Significant ($p < 0.001$) differences were observed among the various cultivars. The lowest hue angles and thus, greater intensities of green were observed in Gymno Carpo (-60.95 in 2018), Fusicaulis (-60.22 in 2019), Meyers (-60.20 in 2018), Messina (-60.01 in 2018) as well as Rossa (-59.90). On the other side of the spectrum, the least saturation of the colour green was observed in Malta (-48.55 in 2019), Fusicaulis (-51.97 in 2018), Algerian (-52.23 in 2019), Robusta (-52.9 in 2019) as well as Fresno (-53.53 in 2018). The majority of the cultivars did not have any significant differences. The greatest intensities of green among the cultivars were observed in 2018 with 15 out of the 20 cultivars having recorded the least hue angles in that year. This proves the observation that was made in the investigation of the effect of the year of harvest on this particular attribute, whereby 2018 was significantly ($p = 0.003$) greater than 2019 (Table 11).

K. *Compressibility and firmness*

In Table 16, the means of the textural and viscosity attributes of the 20 different nopalito cultivars are observed and how they were affected by the cultivar and the year of harvest interaction.

Table 16: Analysis of variance (ANOVA) for the effect of cultivar and the year of harvest interaction on the physical properties of nopalitos

Cultivar	**Year**	**Compressibility in mm**	**Firmness in mm**	**Viscosity (cP)**	**Line-spread (cm)**
Gymno Carpo	2018	$24.33^{abcdefgh}$ ± 6.31	59.94 ± 11.47	21.68^{abc} ± 15.40	5.70^{hi} ± 0.39
	2019	$44.33^{defghijk}$ ± 12.19	77.39 ± 3.57	156.85^{efghi} ± 38.89	$5.00^{cdefghi}$ ± 0.30
R1251	2018	$33.67^{abcdefghijk}$ ± 12.53	62.00 ± 3.74	16.02^{ab} ± 6.62	$4.56^{abcdefgh}$ ± 0.47
	2019	$42.50^{cdefghijk}$ ± 14.94	82.86 ± 7.46	199.98^{fghij} ± 111.41	3.93^{abcd} ± 0.18
Meyers	2018	14.50^{ab} ± 6.44	57.00 ± 14.64	14.78^{ab} ± 2.82	5.42^{ghi} ± 0.26
	2019	$39.17^{bcdefghijk}$ ± 8.95	71.00 ± 8.59	191.72^{fghi} ± 83.94	$4.81^{bcdefghi}$ ± 0.12
Skinners Court	2018	15.83^{abc} ± 7.70	45.72 ± 9.15	25.89^{abc} ± 8.13	4.06^{abcde} ± 0.60
	2019	$44.53^{defghijk}$ ± 15.15	73.78 ± 13.01	43.71^{abcd} ± 19.02	3.92^{abc} ± 0.16
Algerian	2018	47.67^{fghijk} ± 14.61	72.17 ± 6.37	33.73^{abc} ± 12.74	3.60^{a} ± 0.01
	2019	53.67^{ijk} ± 11.18	78.36 ± 6.38	329.34^{k} ± 8.51	$4.74^{bcdefghi}$ ± 0.47
Ofer	2018	$23.33^{abcdefgh}$ ± 12.85	64.00 ± 4.56	19.28^{ab} ± 4.59	3.85^{ab} ± 0.01
	2019	$46.17^{efghijk}$ ± 22.09	74.52 ± 9.54	115.76^{cdefg} ± 90.25	$4.45^{abcdefg}$ ± 0.37
Turpin	2018	20.50^{abcdef} ± 6.02	63.67 ± 11.45	15.61^{ab} ± 6.47	5.45^{ghi} ± 0.29

	2019	$53.50^{ijk} \pm 9.93$	69.33 ± 4.84	$294.21^{jk} \pm 63.60$	$4.87^{bcdefghi} \pm 0.43$
Messina	2018	$31.67^{abcdefghij} \pm 8.82$	54.89 ± 13.20	$35.23^{abc} \pm 2.54$	$4.27^{abcdef} \pm 0.15$
	2019	$48.83^{ghijk} \pm 19.83$	73.88 ± 10.33	$337.67^{k} \pm 23.35$	$4.06^{abcde} \pm 0.64$
Santa Rossa	2018	$25.33^{abcdefgh} \pm 4.63$	55.83 ± 4.98	$27.33^{abc} \pm 25.75$	$4.22^{abcdef} \pm 0.31$
	2019	$47.17^{fghijk} \pm 11.89$	66.78 ± 19.01	$173.36^{fghi} \pm 94.51$	$5.00^{cdefghi} \pm 0.71$
Berg X Mexican	2018	$20.00^{abcdef} \pm 6.96$	60.56 ± 3.72	$18.75^{ab} \pm 8.53$	$5.53^{ghi} \pm 0.40$
	2019	$45.11^{efghijk} \pm 24.06$	71.14 ± 4.91	$204.07^{ghij} \pm 91.75$	$4.23^{abcdef} \pm 0.30$
R1260	2018	$23.17^{abcdefgh} \pm 11.57$	54.39 ± 9.42	$17.59^{ab} \pm 7.48$	$5.31^{fghi} \pm 1.00$
	2019	$53.33^{ijk} \pm 13.22$	75.37 ± 9.28	$108.24^{bcdef} \pm 46.43$	$5.14^{efghi} \pm 0.86$
Fresno	2018	$10.50^{a} \pm 5.54$	46.47 ± 11.53	$19.66^{ab} \pm 6.86$	$4.86^{bcdefghi} \pm 0.72$
	2019	$42.77^{cdefghijk} \pm 11.30$	55.86 ± 24.07	$245.18^{ijk} \pm 23.72$	$5.84^{i} \pm 0.50$
Fusicaulis	2018	$21.33^{abcdefg} \pm 10.39$	44.06 ± 10.07	$53.38^{abcd} \pm 27.47$	$5.06^{defghi} \pm 0.46$
	2019	$53.33^{ijk} \pm 17.53$	67.01 ± 15.33	$49.80^{abcd} \pm 7.61$	$4.60^{abcdefgh} \pm 0.82$
Malta	2018	$26.67^{abcdefghi} \pm 15.02$	55.28 ± 9.09	$19.83^{ab} \pm 6.76$	$3.89^{abc} \pm 0.01$
	2019	$49.17^{ghijk} \pm 20.68$	73.42 ± 4.76	$192.27^{fghi} \pm 52.89$	$4.10^{abcde} \pm 0.39$
Morado	2018	$20.83^{abcdef} \pm 7.78$	62.22 ± 11.47	$12.87^{a} \pm 8.85$	$4.95^{bcdefghi} \pm 0.76$
	2019	$55.17^{jk} \pm 11.72$	73.42 ± 6.58	$133.93^{defgh} \pm 19.18$	$4.67^{abcdefgh} \pm 0.92$
Nudosa	2018	$38.00^{abcdefghijk} \pm 12.99$	43.78 ± 23.12	$13.47^{ab} \pm 6.20$	$4.39^{abcdefg} \pm 0.71$
	2019	$42.67^{cdefghijk} \pm 10.88$	40.11 ± 32.90	$213.19^{hij} \pm 57.19$	$5.35^{fghi} \pm 0.55$
Robusta	2018	$24.17^{abcdefgh} \pm 9.00$	49.78 ± 28.48	$25.04^{abc} \pm 4.35$	$4.66^{abcdefgh} \pm 0.32$
	2019	$53.47^{ijk} \pm 12.89$	51.67 ± 37.69	$62.82^{abcde} \pm 57.36$	$4.67^{abcdefgh} \pm 0.37$
Zastron	2018	$19.00^{abcde} \pm 4.94$	53.83 ± 5.27	$18.66^{ab} \pm 2.54$	$4.75^{bcdefghi} \pm 0.32$
	2019	$50.67^{hijk} \pm 9.42$	69.22 ± 8.12	$322.26^{k} \pm 22.09$	$4.57^{abcdefgh} \pm 0.56$
Rossa	2018	$27.50^{abcdefghij} \pm 5.96$	59.11 ± 8.75	$23.37^{abc} \pm 7.80$	$4.22^{abcdef} \pm 0.44$
	2019	$43.83^{defghijk} \pm 5.08$	64.75 ± 27.21	$160.40^{fghi} \pm 46.43$	$4.77^{bcdefghi} \pm 0.39$
Sicilian Indian Fig	2018	$17.17^{abcd} \pm 6.15$	52.67 ± 5.20	$27.33^{abc} \pm 10.03$	$4.45^{abcdefg} \pm 0.44$
	2019	$59.83^{k} \pm 12.32$	80.50 ± 7.44	$191.19^{fghi} \pm 21.16$	$4.60^{abcdefgh} \pm 0.45$
Average		36.36	62.69	104.64	4.66
Sign. Level		$p = 0.011$	$p = 0.420$	$p < 0.001$	$p < 0.001$

Means with different superscripts in the same column differ significantly

Significant ($p = 0.011$) differences were observed in the compressibility ($p = 0.011$) of the nopalito cultivars over the two years (Table 16). The compressibility of the nopalitos ranged between 10.50 mm (Fresno in 2018) and 59.83 mm (Sicilian Indian Fig in 2019), with an average of 36.36 mm. Generally, the 2018 harvest produced nopalitos which were harder to compress and thus possessed more hardened tissue as compared to the nopalitos that were produced in 2019, with all cultivars having recorded lower values in 2018 than in 2019 (Table 16). Fresno (10.50 mm), Meyers (14.50 mm), Skinners Court (15.83 mm), Sicilian Indian Fig (17.17 mm) and Zastron (19.00 mm) recorded the least compressible nopalitos in 2018. The compressibility of these same cultivars showed to be significantly higher in 2019 as compared to 2018. The cultivars recorded the following measurements of compressibility in 2019: Fresno (42.77 mm), Meyers (39.17 mm), Skinners Court (44.53 mm), Sicilian Indian Fig (59.83 mm) and Zastron (50.67 mm). This further proves the observation that was made in Table 11, in that the effect of the year of harvest had significant ($p < 0.001$) differences on this particular attribute. Additional cultivars which were easily compressed included Algerian (53.67 mm in 2019), Turpin (53.50 mm in 2019), Fusicaulis (53.33 mm in 2019), Morado (55.17 mm in 2019) and Robusta (50.67 mm in 2019). These six aforementioned cultivars were the most compressible in addition to Sicilian Indian Fig (59.83 mm), all of which were recorded in 2019.

The firmness of the various cultivars ranged between 40.11 mm (Nudosa) and 82.86 mm (R1251), with an average of 62.69 mm (Table 16). No significant ($p = 0.420$) differences were observed among the nopalitos over the two years. The 2018 harvest produced nopalitos that were firmer than those produced in 2019, with 19 out of the 20 cultivars having recorded lower measurements in 2018 as compared to 2019. Nudosa was the least flexible cultivar in both years, having recorded a firmness of 43.7 mm in 2018 and 40.11 mm in 2019. Interestingly enough, Nudosa was the only cultivar which was less firm in 2019 compared to 2018. The most firm nopalitos in general, included Nudosa (43.77 mm in 2018 and 40.11 mm in 2019), Fusicaulis (44.06 mm in 2018), Skinners Court (45.72 mm in 2018), Fresno (46.47 mm in 2018) and Robusta (49.78 mm in 2018). On the other side of the spectrum, the least firm and therefore, the most flexible cultivars were R1251 (82.56 mm in 2019), Sicilian Indian Fig (80.50 mm in 2019), Gymno Carpo (77.39 mm in 2019), Algerian (78.36 mm in 2019), R1260 (75.37 mm in 2019) and Messina (73.88 mm in 2019).

L. Viscosity of the extracted mucilage

The two methods that were used to investigate the viscosity of the extracted mucilage from the various cultivars showed significant ($p < 0.001$) differences (Table 16). Looking at the viscosity results obtained using a viscometer, one can deduce that drastic differences were noted in all cultivars, with the 2018 harvest having produced the least viscous nopalitos as compared to the 2019 harvest. The viscosity of the different cultivars ranged between 12.87 cP (Morado) and 337.67 cP (Messina), with an average of 104.64 cP. The lowest viscosities were observed in Morado (12.87 cP), Meyers (14.75 cP), Nudosa (13.47 cP), Turpin (15.61 cP), R1251 (16.02 cP), R1260 (17.59 cP), Zastron (18.66 cP), Berg X Mexican (18.75 cP), Ofer (19.28 cP), Fresno (19.66 cP) and Malta (19.83 cP). All these observations were made in the nopalitos which were harvested in 2018, proving the observation that was made in the investigation of the effect of the year of harvest on this attribute to be true as 2019 produced nopalitos which were significantly ($p < 0.001$) more viscous than those from 2018. On the other side of the spectrum, the most viscous cultivars, which were all recorded in 2019, were Messina (337.67 cP), Zastron (322.26 cP), Algerian (329.34 cP), Turpin (294.21), Fresno (245.18 cP), Nudosa (213.19), Berg X Mexican (204.07 cP), R1251 (199.98 cP), Malta (192.27 cP), Meyers (191.72 cP), Sicilian Indian Fig (191.19 cP), Santa Rossa (173.36 cP), Rossa (160.64 cP), Gymno Carpo (156.85 cP), Morado (133.93 cP), Ofer (115.76 cP) as well as R1260 (108.24 cP). These viscosities accounted for 85% of the cultivars in that year of harvest. This, in turn, means that 17 out of the 20 cultivars which were harvested in 2019 make for a good addition to solutions to provide great viscosity at low concentrations, especially Messina, Algerian and Zastron. The results observed clearly show the extent to which the degree changes in temperature truly affect the viscosity of the cultivars. Interestingly enough, when investigating the effect of cultivar on this attribute (Table 7), Messina, Algerian, Zastron, Turpin, Fresno, Nudosa, Berg X Mexican, Sicillian Indian Fig, R1251, Malta, Meyers and Santa Rossa yielded the highest viscosities, which are all the cultivars that were observed to be the most viscous in 2019 under this investigation. Furthermore, Skinners Court which yielded the least viscous mucilage in Table 7, also yielded relatively low levels of viscosity in this investigation with a reading of 25.89 cP in 2018 and 43.71 cP in 2019.

The line-spread readings of the mucilage that was extracted from the various nopalito cultivars over the two years, ranged between 3.60 cm (Algerian) and 5.84 cm (Fresno) with an average of 4.66 cm. The cultivars that travelled the furthest on the surface and were thus significantly ($p < 0.001$) less viscous compared to other cultivars, are Fresno (5.84 cm in 2019), Gymno Carpo (5.00 cm in 2018 and 5.70 cm in 2019), Berg X Mexican (5.53 cm in 2018), Turpin (5.45 cm in 2018), Meyers (5.42 cm in 2018), Nudosa (5.35 cm in 2019), R1260 (5.31 cm in 2018 and 5.14 cm in 2019), Fusicaulis (5.06 cm in 2018), as well as Santa Rossa (5.00 cm in 2019).

The most viscous cultivars that were observed in this study are Algerian (3.60 cm in 2018), Ofer (3.85 cm in 2018), Malta (3.89 cm in 2018) and Skinners Court (3.93 cm in 2019). Generally, the nopalitos which were harvested in 2018 were slightly less viscous than those harvested in 2019 as 12 out of the 20 cultivars, recorded higher distances travelled on the surface in 2018 than in 2019. This observation, in turn, correlates with the observation that was made when investigating the same parameter using a viscometer, which showed the 2019 harvest to have produced more viscous nopalitos than those harvested in 2018. Interestingly enough, when investigating the effect of cultivar on this particular attribute (Table 7), similar trends were observed. Algerian, Ofer, Malta and Skinners Court were among the most highly viscous cultivars, while Fresno, Gymno Carpo, Turpin and Meyers were among the least viscous cultivars observed.

3.4.5.3. The effect of cultivar and the year of harvest interaction on the physico-chemical and chemical attributes of nopalitos

In Table 17, the mean physico-chemical and chemical attributes of the 20 different nopalitos cultivars are observed and how they were affected by the cultivar and the year of harvest interaction.

Table 17: Analysis of variance (ANOVA) for the effect of cultivar and the year of harvest interaction on the physico-chemical and chemical properties of nopalitos

Cultivar	**Year**	**pH**	**%Titratable acidity expressed as citric acid**	**Sugar (TSS, °Brix)**	**Moisture content (%)**	**% Solids**
Gymno Carpo	2018	4.13 ± 0.16	0.55^{lmn} ± 0.02	5.49^{abcde} ± 0.39	77.47 ± 24.75	22.53 ± 24.75
	2019	4.31 ± 0.29	0.22^{abcde} ± 0.01	$6.04^{abcdefgh}$ ± 0.99	77.54 ± 20.63	22.46 ± 20.63
R1251	2018	3.98 ± 0.13	0.50^{jklmn} ± 0.02	7.60^{hi} ± 0.95	79.27 ± 13.00	20.73 ± 13.00
	2019	4.44 ± 0.09	0.34^{fghi} ± 0.04	$6.35^{bcdefgh}$ ± 1.05	89.12 ± 7.44	10.88 ± 7.44
Meyers	2018	3.99 ± 0.05	0.47^{jklm} ± 0.03	5.57^{abcdef} ± 0.29	90.63 ± 5.91	9.37 ± 5.91
	2019	4.43 ± 0.09	0.32^{efgh} ± 0.05	5.17^{abc} ± 0.23	92.86 ± 14.96	7.14 ± 14.96
Skinners Court	2018	4.18 ± 0.06	0.17^{abc} ± 0.06	5.66^{abcdef} ± 1.37	83.62 ± 3.07	16.38 ± 3.07
	2019	4.24 ± 0.18	0.13^{a} ± 0.01	$5.77^{abcdefg}$ ± 1.33	86.98 ± 5.33	13.02 ± 5.33
Algerian	2018	4.03 ± 0.15	0.45^{ijklm} ± 0.01	5.25^{abc} ± 0.01	92.49 ± 0.42	7.51 ± 0.42
	2019	4.24 ± 0.24	0.39^{ghij} ± 0.01	$6.03^{abcdefgh}$ ± 1.13	87.66 ± 1.93	12.34 ± 1.93
Ofer	2018	4.06 ± 0.09	0.46^{jklm} ± 0.01	5.70^{abcdef} ± 0.01	81.82 ± 12.25	18.18 ± 12.25
	2019	4.32 ± 0.23	0.26^{bcdef} ± 0.08	$5.79^{abcdefg}$ ± 0.15	86.71 ± 2.15	13.29 ± 2.15
Turpin	2018	3.90 ± 0.16	0.60^{n} ± 0.01	8.23^{i} ± 0.63	96.26 ± 39.84	3.74 ± 39.84
	2019	4.14 ± 0.09	0.53^{klmn} ± 0.11	$6.42^{bcdefgh}$ ± 0.55	86.71 ± 2.45	13.29 ± 2.45
Messina	2018	4.13 ± 0.14	0.56^{lmn} ± 0.05	$5.87^{abcdefg}$ ± 0.22	82.71 ± 5.18	17.29 ± 5.18

	2019	4.36 ± 0.18	0.32^{efgh} ± 0.01	6.17^{abcdefgh} ± 0.79	87.63 ± 16.82	12.37 ± 16.82
Santa Rossa	2018	4.10 ± 0.18	0.44^{hijkl} ± 0.08	7.16^{efghi} ± 0.65	87.14 ± 2.34	12.86 ± 2.34
	2019	4.26 ± 0.16	0.18^{abcd} ± 0.03	5.15^{abc} ± 0.99	85.37 ± 56.66	14.63 ± 56.66
Berg X Mexican	2018	4.01 ± 0.06	0.48^{jklmn} ± 0.05	7.00^{defghi} ± 0.38	89.54 ± 1.92	10.46 ± 1.92
	2019	4.02 ± 0.12	0.29^{cdefg} ± 0.04	6.33^{bcdefgh} ± 1.31	86.93 ± 1.61	13.07 ± 1.61
R1260	2018	3.92 ± 0.14	0.27^{bcdef} ± 0.05	6.54^{bcdefgh} ± 0.79	90.60 ± 1.20	9.40 ± 1.20
	2019	4.29 ± 0.17	0.30^{defg} ± 0.01	6.00^{abcdefgh} ± 0.58	88.02 ± 4.99	11.98 ± 4.99
Fresno	2018	4.00 ± 0.14	0.53^{klmn} ± 0.09	7.43^{ghi} ± 0.90	85.10 ± 3.93	14.90 ± 3.93
	2019	4.31 ± 0.16	0.22^{abcdef} ± 0.02	5.28^{abc} ± 0.34	82.31 ± 58.39	17.69 ± 58.39
Fusicaulis	2018	4.01 ± 0.03	0.39^{ghij} ± 0.04	4.58^{a} ± 0.38	87.87 ± 0.26	12.13 ± 0.26
	2019	4.34 ± 0.20	0.21^{abcde} ± 0.04	5.82^{abcdefg} ± 1.31	87.99 ± 2.15	12.01 ± 2.15
Malta	2018	4.07 ± 0.08	0.45^{ijklm} ± 0.01	5.05^{abc} ± 0.01	91.04 ± 1.57	8.96 ± 1.57
	2019	4.32 ± 0.21	0.30^{defg} ± 0.11	6.42^{bcdefgh} ± 0.89	86.93 ± 1.12	13.07 ± 1.12
Morado	2018	4.04 ± 0.28	0.49^{jklmn} ± 0.07	6.48^{bcdefgh} ± 0.55	83.71 ± 12.99	16.29 ± 12.99
	2019	4.22 ± 0.25	0.17^{abc} ± 0.01	6.02^{abcdefgh} ± 0.71	88.31 ± 0.76	11.69 ± 0.76
Nudosa	2018	3.99 ± 0.13	0.42^{hijk} ± 0.11	4.88^{ab} ± 0.39	86.90 ± 3.66	13.10 ± 3.66
	2019	4.24 ± 0.09	0.15^{ab} ± 0.01	4.89^{ab} ± 0.36	85.83 ± 3.17	14.17 ± 3.17
Robusta	2018	4.09 ± 0.10	0.48^{jklmn} ± 0.11	5.53^{abcde} ± 0.91	86.58 ± 2.49	13.42 ± 2.49
	2019	4.33 ± 0.18	0.22^{abcdef} ± 0.03	6.37^{bcdefgh} ± 1.03	87.84 ± 4.55	12.16 ± 4.55
Zastron	2018	4.09 ± 0.18	0.49^{jklmn} ± 0.06	7.23^{fghi} ± 0.32	84.00 ± 4.65	16.00 ± 4.65
	2019	4.18 ± 0.27	0.25^{bcdef} ± 0.02	6.27^{bcdefgh} ± 0.28	87.02 ± 1.20	12.98 ± 1.20
Rossa	2018	4.20 ± 0.16	0.54^{klmn} ± 0.06	6.17^{abcdefgh} ± 0.60	83.30 ± 5.43	16.70 ± 5.43
	2019	4.16 ± 0.35	0.21^{abcde} ± 0.02	7.15^{efghi} ± 0.36	87.76 ± 1.14	12.24 ± 1.14
Sicilian Indian Fig	2018	3.96 ± 0.08	0.57^{mn} ± 0.05	6.57^{cdefghi} ± 0.40	84.56 ± 2.20	15.44 ± 2.20
	2019	4.20 ± 0.34	0.26^{bcdef} ± 0.05	5.39^{abcd} ± 0.66	88.81 ± 1.94	11.19 ± 1.94
Average		4.16	0.36	6.07	86.57	13.43
Sign. level		$p = 0.072$	$p < 0.001$	$p < 0.001$	$p = 0.999$	$p = 0.999$

Means with different superscripts in the same column differ significantly

M. pH

No significant ($p = 0.72$) differences were observed in the effect of the cultivar and the year of harvest interaction on the pH of the different nopalito cultivars over the two years (Table 17). The 2018 harvest generally produced the most acidic nopalitos as compared to the 2019 harvest. Turpin (3.90), R1260 (3.92), Sicilian Indian Fig (3.96), R1251 (3.98), Meyers (3.99) and Nudosa (3.99) were the most acidic cultivars in 2018. Surprisingly enough, these same cultivars recorded higher pH values in 2019, values which were even higher than some cultivars in the study. For example, R1251 (4.44 in 2019) and Meyers (4.43 in 2019) recorded the highest pH contents in 2019 and overall. One explanation for this may be attributed to the fluctuation of electrolytes that takes place on an hourly basis in cladodes which comes with either a drop or rise of pH due to the CAM pathway that is utilised by the plants (Du Toit *et al.*, 2020). Warmer temperatures have been reported to cause a decrease in the pH of cladodes as a result of the presence of a large number of positive ions in cladodes (Du Toit *et al.,* 2020). Negative charges are then neutralised due to the abundance of the hydrogen ions which in turn alter the molecular structure of the mucilage molecules and result in reduced repulsion (Du Toit *et al.,* 2020). The differences in pH of the likes of R1251 and Meyers over the 2 years therefore prove that slight differences and changes that do occur in cultivars over years, highly affect their chemical and physical composition.

N. Moisture content and %solids

The moisture content of the nopalito cultivars ranged between 77.47% (Gymno Carpo) and 96.26% (Turpin), with an average of 86.57% (Table 17). No significant (p = 0.999) differences were observed in the effect of cultivar and the year of harvest interaction on the various nopalitos, over the two years. Apart from Turpin, other cultivars which recorded the highest contents of moisture included Meyers (90.63% in 2018 and 92.86% in 2019), Skinners Court (92.49% in 2018), R1260 (90.60% in 2018), and Malta (91.04% in 2018). When comparing the effect of the cultivar and the year of harvest interaction on the moisture of the nopalitos (Table 17) as well as the effect of the cultivar on the moisture of the nopalitos (Table 9), one can deduce that Gymno Carpo yielded the lowest moisture content in both years. In contrast, Meyers, Turpin and R1260 yielded the highest contents of moisture in both investigations. Albeit, Gymno Carpo yielded the lowest moisture content; it is still relatively high and proves that nopalitos mainly consist of water.

No significant (p = 0.999) differences were observed in the investigation of the effect of cultivar and the year of harvest interaction on the %solids of the various nopalitos over the two years (Table 17). The %solids of the nopalitos ranged between 3.74% (Turpin) and 22.53% (Gymno Carpo), with an average of 13.43%. In general, a greater amount of %solids were observed in 2018 as compared to 2019 with 12 out of the 20 cultivars having recorded higher levels of %solids in 2018. These cultivars were Gymno Carpo (22.53%), R1251 (20.73%), Meyers (9.37%), Skinners Court (16.38%), Ofer (18.18%), Messina (17.29%), Fusicaulis (12.13%), Morado (16.29%), Robusta (13.42%), Zastron (16.00%) and Rossa (16.70%). The least amount of %solids were observed in Turpin (3.74% in 2018), Meyers (7.14% in 2019), Algerian (7.51% in 2018) and Malta (8.96% in 2018). When comparing the results obtained under this investigation to the investigation of the effect of the cultivars on this attribute, one can notice similar trends. For example, Turpin, Meyers and Algerian were some of the cultivars which contained the least amount of %solids while Gymno Carpo yielded the highest (Table 9). Furthermore, the results obtained under this investigation correlate with the observation that was made in Table 12, in that the effect of the year of harvest on the %solids of the various nopalitos was more prevalent in 2018 as compared to 2019.

O. Sugar content (TSS, °Brix)

Sugar readings of the nopalitos over the two years ranged between 4.58 °Brix (Fusicaulis) and 8.23 °Brix (Turpin), with an average of 6.07 °Brix (Table 17). Turpin (8.23 °Brix in 2018), R1251 (7.60 °Brix in 2018), Fresno (7.43 °Brix in 2018), Zastron (7.23 °Brix in 2018), Santa Rossa (7.16 °Brix in 2018), Rossa (7.15 °Brix in 2019) as well as Berg X Mexican (7.00 °Brix in 2018) all showed to have significantly ($p < 0.001$) higher °Brix readings as compared to all cultivars over the two years. On the other side of the spectrum, Fusicaulis (4.58 °Brix in 2018) and Nudosa (4.88 °Brix in 2018 and 4.89 °Brix in 2019), recorded the lowest °Brix readings, which implies that they presented the lowest sugar content. No significant differences were observed between R1251 (6.35 °Brix in 2019), Turpin (6.42 °Brix in 2019), Berg X Mexican (6.33 °Brix in 2019), R1260 (6.54 °Brix in 2018), Malta (6.42 °Brix in 2019), Morado (6.48 °Brix in 2018), Robusta (6.37 °Brix in 2019) and Zastron (6.27 °Brix in 2019). Ten cultivars were observed to contain a higher sugar content in 2018 as compared to 2019, while another 10 of the cultivars were observed to have had a higher sugar content in 2019 as opposed to 2018. Therefore, in as much as significant differences were observed among the cultivars and their effect on this particular attribute, much difference was not observed in terms of the effect of the years of harvest on the sugar content. This supports the observation that was made in the investigation of the effects of the year of harvest on this attribute, which showed no significant ($p = 0.55$) differences present between 2018 and 2019 (Table 12).

P. Titratable acidity

The titratable acidity that was observed from the various nopalito cultivars ranged between 0.13% expressed citric acid (Skinners Court) and 0.60% expressed as citric acid (Turpin), with an average of 0.36% expressed as citric acid (Table 13). Significant ($p < 0.001$) differences were observed among the cultivars, over the two years. When comparing the two years, one can deduce that 2018 produced nopalitos which contained more acid as compared to 2019. This supports the observation that was made in the investigation of the effects of the year of harvest on this attribute, which showed the 2018 harvest to have had a significantly higher titratable acidity content as compared to the 2019 harvest (Table 12). The highest content of acid was observed in Turpin (0.60% expressed as citric acid in 2018 and 0.53% expressed as citric acid in 2019), Sicilian Indian Fig (0.57% expressed as citric acid in 2018), Messina (0.56% expressed as citric acid in 2018), Gymno Carpo (0.55% expressed as citric acid in

2018), Rossa (0.54% expressed as citric acid in 2018), Fresno (0.53% expressed as citric acid in 2018) and R1251 (0.50% expressed citric acid in 2018). Skinners Court (0.17% expressed as citric acid in 2018 and 0.13% expressed as citric acid in 2019), Morado (0.17% expressed as citric acid in 2019) and Nudosa (0.15% expressed as citric acid in 2019) reported the lowest titratable acidity (significant $p < 0.001$) as compared to all the cultivars in the study. To understand the modifications that occur in the titratable acidity content of nopalitos that are a result of the time of harvest, Corrales-García *et al.* (2004) studied the changes in the acidity of Milpa Alta variant nopalitos from the time of harvest and the elapsed time since harvest. The investigation observed variations in the titratable acidity content of nopalitos that were harvested between 6:00h and 14:00h . In this period, 50% of the acidity diminished. In contrast, a rapid increase in acidity content was observed in the first period of natural darkness. On the second day of observations, similar trends were reported which also largely depended on the lengths of daylight and darkness. All these observed changes were attributed to the effects of the CAM metabolism of the nopalitos. This may also explain the changes in the titratable acidity of the nopalitos that were harvested over the two years in this study.

3.5. Summary

Interesting trends were noticed in the effect of the cultivar, the year of harvest as well as the cultivar and the year of harvest interaction on the various morphological, physiological, physical, physico-chemical and chemical attributes of the nopalitos. From the different treatments that were determined by ANOVA, the effect of cultivar on the various parameters showed to have the most significant differences when comparing the 20 cultivars. The only parameters that were not significantly affected by the treatments under this category were pH, compressibility, moisture content, % solids, % waste, as well as the viscosity of the nopalitos. The reported results do show that various factors contribute to the overall composition and therefore, the quality of nopalitos. It is known that the ideal commercial raw nopalito must be thin, turgid, look fresh in appearance, have a bright green colour, and be preferably low in acidity and mucilage content as these two characteristics have a negative influence on the eating qualities of the vegetable. Taking that into consideration, the below conclusions can be made. Under the investigation of the effect of cultivar on the various properties, the average weight of the nopalitos was 88.96 g with Ofer (60.07 g) and Morado (63.97 g) being the least heavy cultivars recorded, while Fresno (155.60 g), Santa Rossa (109.61 g), Fusicaulis (149.53 g), Robusta (151.03 g) and Nudosa (153.13 g) were the heaviest recorded cultivars. The average length of the nopalitos was 17.20 cm with the shortest cultivars being Berg X Mexican (15.99 cm) and Gymno Carpo (16.03 cm), while the longest cultivars that were recorded were Santa Rossa (19.63 cm), Fresno (19.39 cm) and Nudosa (19.37 cm). Thin nopalitos are the most preferred by consumers; as such, this parameter is a critical determinant of establishing which cultivars are of great significance. The average diameter of the cultivars of this study was 0.55 cm with the thickest cultivars being Fusicaulis (0.96 cm), Fresno (0.89 cm), Robusta (0.88 cm) and Nudosa (0.84 cm). Interestingly enough, these same cultivars were observed as being the heaviest in terms of weight. On the other side of the spectrum, the thinnest cultivars that were observed were Santa Rossa (0.42 cm), Gymno Carpo (0.43 cm), Morado (0.43 cm), R1251 (0.44 cm) as well as Ofer (0.44 cm).

Berg X Mexican (4.01) and Turpin (4.02) showed to be the most acidic cultivars, as they recorded the lowest pH levels. Messina and Gymno Carpo yielded the highest pH levels and were thus the least acidic cultivars in the study. The average compressibility and firmness of the cultivars were 36.36 mm and 62.69 mm, respectively. The most firm cultivars were Nudosa (41.94 mm) and Robusta (50.72 mm), while the most flexible cultivars were Algerian (75.26 mm) and R1251 (72.43 mm). The cultivars which had the softest tissue were Algerian (50.67 mm) and Nudosa (40.33 mm), while the hardest tissue was observed in Fresno (26.64 mm) as well as Meyers (26.83 mm). Nopalitos are appreciated for their high moisture content whose average amounted to 86.59% in this investigation. The highest moisture content was observed in Meyers (91.74%), Turpin (91.49%) and Algerian (90.08%), while the least moisture was observed in Gymno Carpo (77.51%) and Fresno (83.70%). The %Solids of the cultivars recorded an average of 13.43%, with the highest content having been observed in Gymno Carpo (22.49%) and Fresno (16.30%), while the lowest content was observed in Meyers (8.26%) as well as Turpin (8.51%). The most amount of mucilage which was extracted was from Fusicaulis (69.63 g), while the least amount of mucilage was obtained from Ofer (7.87 g). The average mucilage %yield was 20.84%. When comparing the two methods that were used to investigate the viscosity of the various nopalitos, the line-spread method was shown to produce the most quantifiable results as reported by Du Toit (2017). Furthermore, it was necessary to determine the viscosity of the nopalitos as it was reported that greater viscosities in food have appetite-suppressing capabilities, therefore, reducing food intake (Marciani *et al.,* 2001; Mattes & Rothacker, 2001). Using a viscometer, the highest viscosity was observed in Messina (186.45 cP) and Zastron (177.46 cP). The least viscous cultivars in terms of the line-spread test method were Fresno (5.35 cm) and Gymno Carpo (5.35 cm). The average titratable acidity expressed as citric acid was 0.37% with Turpin (0.57% expressed as citric acid) being the highest while Skinners Court (0.15% expressed as citric acid) was the lowest. The total soluble solids readings of the nopalitos showed an average of 6.07 °Brix with Turpin (7.32 °Brix) yielding the highest sugar content, while Nudosa (4.89 °Brix) was the lowest. Gymno Carpo was the lightest (L* = 53.38) cultivar, with the greenest tone (a* = -20.16), the most yellow-green tone (b* = 34.30) as well as brightness (C* = 39.81). Fusicaulis and Fresno had the least lightness, green tone, and yellow tone as well as saturation. Malta recorded the highest (-53.99) hue angle, while Rossa recorded the lowest hue angle.

The effect of the year of harvest on the various properties of the nopalitos seemed to have a bigger effect on the nopalitos which were harvested in 2019 as compared to 2018. The acidity and pH of the samples were generally higher in 2019 than in 2018. This is because, the pH values in 2018 were lower than those recorded in 2019, which means that 2018's nopalitos were more acidic than those from 2019. The titratable acidity expressed as % citric acid was also higher in 2018 than in 2019, which means that 2018's nopalitos also had higher acidity levels than the latter's nopalitos. With regards to the sugar content, the nopalitos which were harvested in 2019 contained a lower sugar content than those that were harvested in 2018 overall, which is a desirable trait to reduce the impact of the acidity present in the nopalitos. Nopalitos are appreciated for their high moisture content which makes them a low-calorie vegetable. The moisture content of the nopalitos was observed to be higher in 2019 than in 2018. With regards to flexibility, the 2018 harvest showed to have produced the least flexible range in the various nopalitos as opposed to the 2019 harvest.

Though the colour coordinates were in more or less similar ranges for both years, the 2018 nopalitos presented higher values than 2019, which translates to the fact that the former had the best colour qualities. In terms of the mucilage that was extracted, there were similar amounts in both years, however, the 2019 nopalitos had more extractable mucilage than in 2018 using the patented extraction method of this study (Du Toit and De Wit, 2011). In fact, it has been reported that the amount of mucilage in nopalitos is the same, and the only difference is brought upon by the amount of mucilage that can be extracted which largely depends on the pH of the mucilage (Du Toit *et al.,* 2019). A lower pH often results in more viscous mucilage which is extracted, as such, the 2018 harvest was better in this aspect as it produced nopalitos with the least viscous slime.

The results obtained under the investigation of the effect of cultivar and the year of harvest interaction also correlated with the results obtained under the aforementioned investigation. It was also interesting to observe that the reported work was easily comparable to commercially world-grown cultivars such as ‘Atlixco’ and ‘Milpa Alta’.

3.6. Conclusion

Overall, the 2018 harvest yielded slightly better quality nopalitos than the 2019 harvest. This is because the nopalitos contained the least viscous mucilage which often interferes with the eating qualities of the nopalitos when highly viscous, they had the best colour qualities, and they had higher contents of sugar and %solids (Table 18). The cultivars of great interest were Meyers, Malta, Nudosa, Fusicaulis, Fresno and Morado which will be included in the next chapter for further studies, including the investigation of their nutritional and sensorial properties. A part of the reason for the selection was a result of the interesting variations that were found in the results of the cultivars which were obtained over the two years.

The reported work has shown the acceptable quality of the nopalitos as a vegetable source to be consumed in South Africa and future research must include extensive comparative analysis between South African cultivars as well as those that have been extensively commercialised in other countries.

Table 18: A summary of the best year of harvest for nopalito attributes

Parameter	Year	
	2018	2019
Mucilage (must be low)	✓	
Diameter (must be low as it signifies how thin the nopalitos are)	✓	
Colour coordinates:		
L* (high value)	✓	
a* (below zero)	✓	
b* (high value)	✓	
Chroma (high value)	✓	
Hue angle (high value)	✓	
Compressibility (high value)		✓
Firmness (High value)		✓
Viscosity using a viscometer (Low value)	✓	
Viscosity using the line-spread method (High value)	✓	✓
pH (High value)		✓
Moisture content (High value)		✓
%Solids (High value)	✓	
Sugar content (High value)	✓	
%Titratable acidity (Low value)		✓

Chapter 4

4. Sensory profiling of nopalitos from 20 cactus pear cultivars, using the Check-All-That-Apply (CATA) technique

Abstract

The study aimed to determine the consumer acceptability and the sensory profile of nopalitos from 20 South African cactus pear cultivars and to compare them to common vegetables i.e., cucumber and green pepper which served as vegetable controls. Sixty-one consumers ranked the overall liking of the samples on a 9-point hedonic scale. The same panellists selected sensory characteristics, which they best associated with certain attributes, by using the Check-all-that-apply (CATA) question. Thirty-two attributes were divided into five categories, namely Colour, appearance, flavour, taste and aftertaste. No significant differences were noted between the hedonic scaling for the nopalito cultivars. There were significant differences (p<0.05) for 25 of the CATA attributes, between the 20 cactus pear cultivars and two control vegetables. Correspondence analysis explained 69.76% of the variation between the samples, while agglomerative hierarchical clustering depicted four consumer preference clusters. External preference mapping indicated the regions, for which consumers exhibited the most liking.

4.1. Introduction

The latest trend that has taken over consumers around the world is the concept of healthy eating, which involves the promotion and increased consumption of healthy, whole, unprocessed foods. This concept dates back to the natural health food movement of the 1960s, whereby processed foods were shunned from a moral and societal opinion, as opposed to issues related to health and nutrition (Welland, 2016). The wave of clean eating has further been perpetuated by bodybuilders, fitness models, as well as media personalities, who have since inspired a new generation of healthy eaters. This has, thus, resulted in various industries looking at ways of developing and manufacturing new “functional food sources that serve as high value-added products for their end-use” (Sáenz, 2000).

In most cases, many of the functional food sources that have been developed, have been those derived from plants, e.g. nopalitos. Nopalitos are young edible joints, which belong to the Cactaceae family of plants, whose genus *Opuntia*, is endemic to Latin American and Mediterranean countries. These young edible joints have formed a major part of the daily diet of these populations, where it is regarded as a staple green vegetable. This vegetable is beneficial, due to its ability to remedy ailments, such as inflammation and viral infections that can be ascribed to its high amounts of nutrients, such as minerals, phenolic compounds, vitamins and betalains (Stintzing and Carle, 2005; Feugang *et al.,* 2006).

In addition to being consumed as vegetables, nopalitos can also be used as industrial hydrocolloids, where they are utilised to alter various eating properties of food, such as taste, appearance, texture and stability (Jun *et al.,* 2013).

Since being exported to Asia and Europe, these stems have also become a traditional staple in many countries, where they are mainly consumed pickled or brined. According to Pérez-Cacho *et al.* (2006), a raw nopalito of good quality must be fresh-looking, bright-green Coloured, thin and turgid. Additional quality factors that have been assessed by consumers include the number of spines, the amount of mucilage, as well as the acidic taste (Sáenz, 2000; Corrales-García *et al.,* 2004). Nopalitos that are low in acidity (Razo & Sánchez, 2002) and mucilage are mostly preferred. According to Pérez-Cacho *et al.* (2006), the common procedure for preparing nopalitos involves the removal of spines; washing; dicing; and scalding/cooking. When cooked, they resemble green beans in flavour (Rodríguez & Cantwell, 1988).

In South Africa (SA), young cladodes are normally fed to livestock, as part of forage. The vegetable's medicinal and health benefits also warrant probing, in order to be accepted as a potential functional food, as consumers are the ultimate determinants of a product's overall success. As such, it is important to conduct sensory tests on nopalitos to provide reputable information, regarding their sensorial characteristics.

The most significant qualities that consumers regard when purchasing food products are the sensory characteristics, which include appearance, aroma, taste and texture (Meilgaard *et al.,* 2006). Moskowitz *et al.* (2012) deem it mandatory to utilise various sensory techniques to apprehend how sensory characteristics of products are perceived by consumers (Hiscock *et al.,* 2018). One such technique is the Check-All-That-Applies (CATA) question, which involves giving consumers descriptive terms, to determine the attributes that best describe the various samples presented to them. The technique is adorned for its simplicity, in terms of the ease at which consumers can complete it, as well as its time efficiency.

No sensory analysis has been carried out on nopalitos in SA. It therefore, becomes critical to investigate the sensory characteristics of the 20 selected South African nopalito cultivars, to determine their overall acceptability by consumers, so that they can be introduced to the country as a staple vegetable.

4.2. Material and methods

4.2.1. Study area, experimental design and plant materials

Six nopalitos, from each of the 20 selected South African cultivars (Table 20), were harvested at a length of 18 cm, one day before sensory analysis. The samples were harvested at a cactus pear research orchard, on one of the campuses of the local university. The 20 cultivars were highlighted by Du Toit (2017), as exhibiting the most potential for consumer consumption in SA, because of their low mucilage production and low acidity.

Several researchers have compared nopalitos to regular green vegetables, such as green beans, green peppers, cucumbers and asparagus, in terms of flavour and taste (Rodríguez and Cantwell, 1988; Razo and Sánchez, 2002; Pérez-Cacho *et al.,* 2006; www.perishablenews.com, 2020). As such, it becomes imperative to investigate how consumers perceive nopalitos, to compare them to these regular green vegetables.

4.2.2 Nopalito processing

Upon harvesting, the nopalito samples were thoroughly washed under running water, dethorned, rinsed again and cut into equal bite-sized cubes, measuring 20 x 20 mm (Figure 8). The samples were stored in air-tight containers at 4°C. Store-bought cucumbers and green peppers were also washed under running water, cut into equal bite-sized pieces and stored at 4°C.

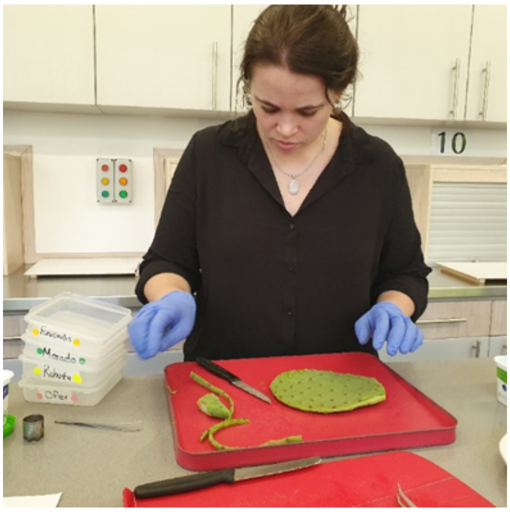

Figure 8: The processing of various harvested nopalito cultivars

4.2.3. Sample preparation for the lexicon test and overall sensory analysis

A lexicon is a set of standardised vocabularies, which are developed and used by highly trained panellists, to describe a broad array of sensory attributes, which are present in a product (Suwonsichon, 2019). To develop a lexicon for this study, 12 highly trained panellists were used to describe the 24 samples (20 nopalito cultivars and four regular green vegetables, namely baby marrow, cucumber, green bean and green pepper). The samples were codified with random three-digit numbers and randomly served to the panel (Figure 9).

The panel was requested to select relevant words, which could be used to best describe the samples in terms of aroma, flavour and aftertaste. Mineral water was provided as a palate cleanser, to ensure that no carryover effects were experienced between the samples, which would otherwise alter the panel’s assessment of the samples. From the lexicon generation, common words were identified for the CATA question. This panel also decided on the use of green pepper and cucumber, as vegetable controls.

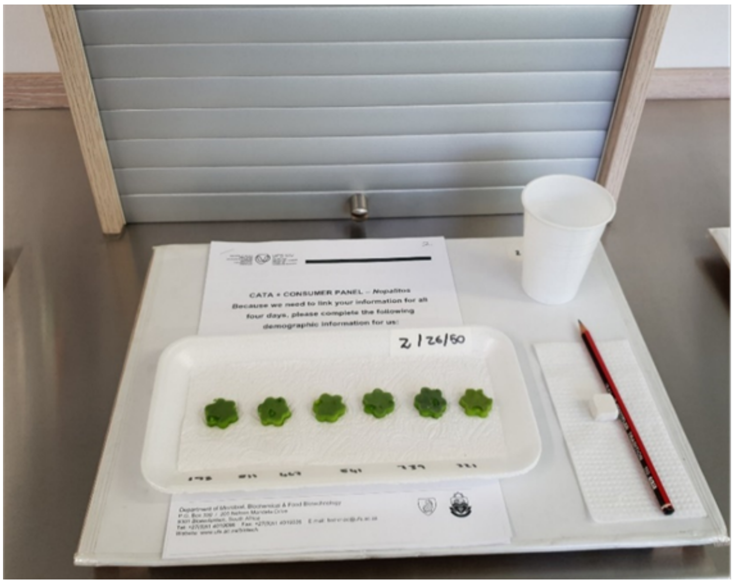

Figure 9: A foam tray filled with various three-digit coded samples for the lexicon test as well as the CATA sensory analysis

4.2.4 Overall acceptability

A consumer test was conducted to investigate the overall acceptability of the 20 nopalito cultivars, cucumber and green pepper. A total of 61 participants (49 females and 11 males) participated, with a 50/50 racial split (black/white) and an age range between 18 and 65 years, suggesting that staff members and students partook (Table 19).

Table 19: Demographic profile and cluster analysis of the 61-member consumer panel, for nopalitos from 20 cactus pear cultivars, cucumber and green pepper

	Overall %	**Cluster 1 %:**	**Cluster 2 %:**	**Cluster 3 %:**	**Cluster 4 %:**
N	60	10	28	11	12
Gender					
Male	18	20	25	0	17
Female	82	80	75	100	83
Race:					
African	34	40	21	27	67
Coloured	5	0	7	9	0
White	61	60	72	64	33
Age (Years)					
18-30	54	40	54	46	75
31-50	30	20	36	36	17
51-65	16	40	10	18	8
Occupation					
Staff	49	50	54	64	25
Student	46	50	43	27	67
Staff and student	5	0	3	9	8

The study was conducted at the sensory facility of the local university and was held over 10 days, to ensure that all consumers were able to taste all samples. The principal criteria for panel selection were availability, willingness to participate and consumption of either cucumber or green pepper or both (at least once a week). To ensure that complete visibility of the samples was achieved while tasting, white lights were used in the sensory laboratory. For overall acceptability, participants were asked to rank the sample on a nine-point hedonic scale, ranging from 1 (dislike extremely) to 9 (like extremely) (Stone & Sidel, 2004) (Table 20).

4.2.5 Check-All-That-Apply

Thirty-two attributes, divided into six categories, were sequentially presented on the ballot, according to the various sensory modalities, i.e., Colour (green), appearance (fresh, thin, thick, slimy), taste (sweet, sour, salty, bitter, savoury/umami), texture (stalky, chewy, fibrous, slimy, hard, spongy, crisp, soft), aftertaste (sweet, sour, bitter, savoury/umami, metallic, none) and flavour (grassy, cucumber, green pepper, green bean, fresh, mild, bland, herbal) (Figure. 3).

Figure 10: CATA analysis in progress

4.2.6 Statistical analysis

In order to analyse the differences among group means in each sample during the hedonic test, an analysis of variance (ANOVA) was done. The Tukey Kramer multiple comparison test ($p \leq 0.05$) was applied if the main effect was significant, so as to determine the direction of the differences between the mean values (Number Cruncher Statistical Systems (NCSS), 2018). For analyses of the CATA data, each term's frequency of use was determined, by counting the number of consumers that used each specific attribute to evaluate the samples, from which a contingency table was created. To determine significant differences ($p \leq 0.05$) of each attribute between the various samples, the Cochran's Q test was used (XLSTAT, 2018). The Marascuilo procedure ($p \leq 0.05$) was applied to determine the direction of the terms used, amidst the various samples. Correspondence analysis (CA), which is a method used to graphically visualise rows and columns of a contingency table encompassing consumers' responses to the CATA question, was used in this study. Furthermore, CA was performed (XLSTAT, 2018) on the various antiphons to the CATA question, to determine the relationships between the attributes and samples, as well as to provide a two-dimensional sensory map of the differences and similarities between the samples and the attributes used to describe them (Ares and Jaeger, 2015). Agglomerative hierarchical clustering (AHC) was administered to analyse the hedonic ratings, to further discern consumers' responses.

The dendrogram, which was obtained from the AHC, was used to establish noticeable clusters and grouped samples based on their sensory characteristics (Hasted, 2018). Automatic truncation was selected, to demonstrate the clusters and to resolve when the stoppage of aggregating observations would take place, taking into consideration the Euclidean distances and Ward's aggregation precedent (XLSTAT, 2018). Lastly, external preference mapping (EPM) was used to tie the consumers' overall liking scores to their responses to the CATA question. Class centroids from AHC, principal coordinates from the CA and a vector model were utilised to create a preference map and counterplot. A preference map is a decision support tool in analyses, where a configuration of objects has been obtained from a first analysis and where a table with complementary data describing the attributes, is available (XLSTAT, 2018). The aforementioned map was superimposed on a contour plot, to show the regions which correspond to the various preference consensus levels on a chart, whose axes are the same as the preference map (XLSTAT, 2018). Cold (blue) regions on the chart represented high preferences with a low proportion of models, while the hot (red) regions represented high preferences with a high proportion of models (XLSTAT, 2018).

4.2.8 Ethical consideration

The Ethics Committee of the Faculty of Natural and Agricultural Sciences, UFS, Bloemfontein approved this study. The ethical clearance number is UFS-HSD2018/0224/0406.

4.3. Results and discussion

4.3.1 Overall acceptability

The ANOVA results from the overall acceptability test are summarised in Table 20, which presents highly significant differences ($p < 0.05$) between cucumber and the nopalitos, as well as between green pepper and the nopalitos. No significant differences were found between the liking of the nopalito cultivars, as well as between green pepper and cucumber. Cucumber was ranked the highest, which corresponded to 'like moderately' on the hedonic scale. The ranking of green pepper placed it in the 'like slightly' category on the hedonic scale. Among the 20 nopalito cultivars, Skinners Court, Turpin, Fusicaulis, R1251 and Rossa were 'neither liked nor disliked' by the consumers, as they were all ranked higher than the other cultivars. The most preferred nopalito cultivar out of the 20 was Skinners Court, as it received the highest ranking as compared to all nopalito cultivars. The remaining 15 nopalito cultivars were 'disliked slightly' by the consumers, with the least liked cultivar being Robusta.

Table 20: Analysis of variance for consumers' overall liking of nopalito samples, from 20 cultivars of cactus pears and two controls, namely cucumber and green pepper

Cultivar	**Overall Liking**
Gymno Carpo	4.56^a ± 1.74
Malta	4.67^a ± 1.89
Messina	4.59^a ± 1.95

Cucumber	$7.13^{b} \pm 1.67$
Meyers	$4.57^{a} \pm 1.65$
Berg & Mexican	$4.70^{a} \pm 1.99$
Nudosa	$4.70^{a} \pm 1.87$
Ofer	$4.52^{a} \pm 1.73$
Robusta	$4.21^{a} \pm 2.00$
Green pepper	$6.64^{b} \pm 1.86$
Morado	$4.59^{a} \pm 1.80$
Fusicaulis	$4.72^{a} \pm 1.66$
Rossa	$5.07^{a} \pm 1.76$
Santa Rossa	$4.89^{a} \pm 1.71$
R1251	$5.07^{a} \pm 1.74$
Skinners Court	$5.20^{a} \pm 1.92$
Fresno	$4.72^{a} \pm 1.65$
R1260	$4.98^{a} \pm 1.77$
Algerian	$4.89^{a} \pm 1.80$
Sicilian Indian Fig	$4.95^{a} \pm 1.80$
Turpin	$5.13^{a} \pm 1.79$
Zastron	$4.53^{a} \pm +2.04$
Sign. Level	$p < 0.05$

4.3.2. Check-all-that-apply

Table 21 depicts a summary of the sensory characteristics of the 22 samples, the frequency of their use, as well as the significant differences between the genotypes. The most frequently used terms were “green colour”, “fresh”, “thick”, “sour”, “salty”, “crispy”, “chewy”, “slimy”, “sour aftertaste”, “grassy flavour” and “fresh flavour”. The least used terms were “slimy appearance”, “sweet taste”, “umami taste”, “fibrous texture”, “hard texture”, “savoury aftertaste”, “mild flavour” and “bland flavour”.

Table 21: Summary of sensory characteristics, frequency of use and significant differences between genotypes for the CATA question

Cultivar	Gymno Carpo	Malta	Messina	Cucumber	Meyers	Berg & Mexican	Nudosa	Ofer	Robusta	Green pepper	Morado	Fusicaulis	Rossa	Santa Rossa	R1251	Skinners Court	Fresno	R1260	Algerian	Sicilian Indian Fig.	Turpin	Zastron	Frequency of use
Green Colour*	54^{ab}	57^{ab}	55^{ab}	49^{a}	57^{ab}	51^{ab}	55^{ab}	55^{ab}	55^{ab}	55^{ab}	58^{ab}	57^{ab}	56^{ab}	59^{b}	59^{b}	56^{ab}	56^{ab}	56^{ab}	56^{ab}	58^{ab}	54^{ab}	56^{ab}	1224
Fresh appearance*	35^{abc}	31^{ab}	32^{ab}	48^{c}	32^{ab}	32^{ab}	30^{ab}	33^{abc}	21^{a}	40^{bc}	34^{abc}	31^{ab}	36^{abc}	34^{abc}	32^{ab}	27^{ab}	29^{ab}	33^{abc}	30^{ab}	31^{ab}	30^{ab}	28^{ab}	709
Thin appearance*	12^{abc}	18^{bcde}	4^{ab}	37^{f}	14^{abc}	17^{abcde}	15^{abcd}	33^{ef}	1^{a}	18^{bcde}	33^{ef}	18^{bcde}	27^{cdef}	27^{cdef}	28^{cdef}	19^{bcde}	11^{abc}	25^{cdef}	32^{def}	16^{abcde}	22^{cdef}	17^{bcde}	444
Thick appearance*	30^{cd}	23^{bcd}	31^{cd}	3^{a}	25^{bcd}	26^{bcd}	31^{cd}	9^{ab}	54^{e}	29^{cd}	9^{ab}	30^{cd}	17^{abc}	13^{abc}	17^{abc}	30^{cd}	39^{de}	21^{abcd}	17^{abc}	29^{cd}	25^{bcd}	30^{cd}	538
Slimy appearance*	6^{abc}	6^{abc}	12^{bc}	2^{ab}	6^{abc}	6^{abc}	5^{ab}	5^{ab}	16^{c}	7^{abc}	4^{ab}	3^{ab}	5^{ab}	5^{ab}	3^{ab}	4^{ab}	2^{ab}	5^{ab}	1^{a}	6^{abc}	1^{a}	3^{ab}	113
Sweet taste*	3^{a}	1^{a}	1^{a}	27^{b}	3^{a}	3^{a}	2^{a}	2^{a}	5^{a}	30^{b}	5^{a}	4^{a}	4^{a}	4^{a}	6^{a}	2^{a}	6^{a}	7^{a}	4^{a}	5^{a}	3^{a}	5^{a}	132
Salty taste*	25^{ab}	21^{ab}	23^{ab}	17^{ab}	22^{ab}	26^{b}	21^{ab}	17^{ab}	17^{ab}	9^{a}	17^{ab}	18^{ab}	17^{ab}	15^{ab}	20^{ab}	25^{ab}	22^{ab}	19^{ab}	15^{ab}	18^{ab}	18^{ab}	16^{ab}	418
Bitter taste^{NS}	13	13	12	3	13	9	11	12	12	7	15	18	12	14	12	12	14	12	11	11	14	12	262
Sour taste*	26^{b}	38^{b}	39^{b}	1^{a}	40^{b}	31^{b}	41^{b}	41^{b}	32^{b}	3^{a}	37^{b}	26^{b}	43^{b}	41^{b}	41^{b}	28^{b}	32^{b}	35^{b}	40^{b}	40^{b}	41^{b}	40^{b}	736
Savoury / Umami taste*	9^{ab}	6^{ab}	3^{a}	13^{ab}	2^{a}	6^{ab}	3^{a}	1^{a}	4^{a}	17^{b}	3^{a}	4^{a}	9^{ab}	3^{a}	6^{ab}	11^{ab}	7^{ab}	8^{ab}	11^{ab}	7^{ab}	6^{ab}	9^{ab}	148
Stalky texture*	9^{ab}	9^{ab}	6^{ab}	4^{a}	8^{ab}	5^{ab}	7^{ab}	11^{ab}	16^{b}	12^{ab}	4^{a}	10^{ab}	6^{ab}	12^{ab}	5^{ab}	11^{ab}	11^{ab}	4^{a}	10^{ab}	5^{ab}	5^{ab}	9^{ab}	179
Chewy texture*	21^{ab}	18^{ab}	22^{ab}	25^{ab}	18^{ab}	22^{ab}	24^{ab}	20^{ab}	12^{a}	24^{ab}	23^{ab}	25^{ab}	20^{ab}	15^{ab}	24^{ab}	22^{ab}	26^{ab}	19^{ab}	21^{ab}	21^{ab}	21^{ab}	30^{b}	473
Fibrous texture*	5^{a}	5^{a}	5^{a}	7^{ab}	4^{a}	4^{a}	8^{ab}	6^{a}	18^{b}	10^{ab}	4^{a}	9^{ab}	6^{a}	6^{a}	8^{ab}	4^{a}	7^{ab}	8^{ab}	7^{ab}	7^{ab}	11^{ab}	13^{ab}	162
Slimy texture*	26^{cd}	22^{cd}	31^{d}	4^{ab}	26^{cd}	24^{cd}	11^{abc}	24^{cd}	33^{d}	1^{a}	26^{cd}	20^{bcd}	24^{cd}	17^{abcd}	25^{cd}	19^{bcd}	23^{cd}	23^{cd}	20^{bcd}	26^{cd}	19^{bcd}	20^{cd}	464
Hard texture*	5^{ab}	4^{a}	6^{ab}	6^{ab}	6^{ab}	5^{ab}	8^{ab}	6^{ab}	17^{b}	12^{ab}	7^{ab}	12^{ab}	4^{a}	4^{a}	9^{ab}	7^{ab}	14^{ab}	6^{ab}	6^{ab}	6^{ab}	6^{ab}	10^{ab}	166
Spongy texture*	13^{b}	13^{b}	13^{b}	5^{ab}	12^{ab}	11^{ab}	11^{ab}	14^{b}	10^{ab}	1^{a}	16^{b}	12^{ab}	17^{b}	13^{b}	14^{b}	10^{ab}	12^{ab}	10^{ab}	7^{ab}	14^{b}	9^{ab}	8^{ab}	245
Crispy texture*	22^{abc}	23^{abcd}	17^{abc}	39^{de}	20^{abc}	21^{abc}	22^{abc}	17^{abc}	8^{a}	50^{e}	14^{ab}	21^{abc}	24^{abcd}	23^{abcd}	19^{abc}	32^{cd}	19^{abc}	20^{abc}	29^{bcd}	26^{bcd}	27^{bcd}	21^{abc}	514
Soft texture*	13^{bc}	9^{abc}	4^{abc}	8^{abc}	17^{c}	9^{abc}	3^{abc}	9^{abc}	10^{abc}	1^{a}	14^{bc}	6^{abc}	9^{abc}	12^{abc}	8^{abc}	9^{abc}	5^{abc}	12^{abc}	11^{abc}	10^{abc}	12^{abc}	7^{abc}	198
Metallic aftertaste^{NS}	3	4	7	3	7	3	3	5	3	3	6	8	6	3	5	6	6	8	7	5	4	3	108
Sweet aftertaste*	3^{a}	1^{a}	3^{a}	22^{b}	5^{a}	4^{a}	2^{a}	3^{a}	2^{a}	22^{b}	9^{a}	3^{a}	1^{a}	2^{a}	2^{a}	4^{a}	4^{a}	7^{a}	3^{a}	5^{a}	1^{a}	7^{a}	115
Sour aftertaste*	23^{b}	31^{b}	30^{b}	2^{a}	32^{b}	28^{b}	34^{b}	36^{b}	30^{b}	2^{a}	32^{b}	31^{b}	33^{b}	30^{b}	34^{b}	25^{b}	27^{b}	26^{b}	25^{b}	29^{b}	38^{b}	32^{b}	610
Bitter aftertaste*	15^{ab}	15^{ab}	10^{ab}	2^{a}	12^{ab}	11^{ab}	11^{ab}	6^{ab}	10^{ab}	9^{ab}	6^{ab}	16^{b}	7^{ab}	14^{ab}	8^{ab}	10^{ab}	11^{ab}	4^{ab}	8^{ab}	9^{ab}	13^{ab}	10^{ab}	217
Savoury aftertaste*	3^{ab}	4^{ab}	1^{a}	10^{b}	1^{a}	3^{ab}	3^{ab}	4^{ab}	4^{ab}	8^{ab}	2^{ab}	6^{ab}	4^{ab}	3^{ab}	2^{ab}	7^{ab}	1^{a}	9^{ab}	4^{ab}	6^{ab}	6^{ab}	6^{ab}	97
No aftertaste^{NS}	8	8	9	10	4	9	11	9	10	9	9	5	11	9	9	12	6	7	10	10	7	5	187
Grassy flavour*	31^{b}	27^{b}	26^{b}	6^{a}	22^{ab}	27^{b}	29^{b}	29^{b}	26^{b}	6^{a}	23^{ab}	29^{b}	25^{b}	18^{ab}	27^{b}	24^{b}	30^{b}	22^{ab}	26^{b}	26^{b}	22^{ab}	24^{b}	525
Cucumber flavour*	8^{a}	13^{a}	7^{a}	58^{b}	9^{a}	9^{a}	5^{a}	9^{a}	6^{a}	4^{a}	8^{a}	9^{a}	6^{a}	7^{a}	6^{a}	8^{a}	8^{a}	10^{a}	6^{a}	6^{a}	9^{a}	6^{a}	217
Green pepper flavour*	8^{a}	8^{a}	13^{a}	2^{a}	10^{a}	7^{a}	9^{a}	8^{a}	6^{a}	53^{b}	12^{a}	2^{a}	5^{a}	4^{a}	6^{a}	7^{a}	5^{a}	8^{a}	3^{a}	8^{a}	7^{a}	10^{a}	201
Green bean flavour*	13^{abc}	13^{abc}	7^{abc}	1^{a}	15^{bc}	15^{bc}	12^{abc}	9^{abc}	14^{abc}	2^{ab}	9^{abc}	15^{bc}	11^{abc}	12^{abc}	11^{abc}	10^{abc}	14^{abc}	13^{abc}	13^{abc}	10^{abc}	16^{c}	6^{abc}	241
Fresh flavour^{NS}	17	18	20	28	14	15	17	16	17	19	21	16	17	20	17	16	12	18	17	17	16	17	385
Mild flavour^{NS}	8	8	1	6	4	9	6	8	4	2	5	8	5	10	3	8	7	9	8	7	6	8	140
Bland flavour^{NS}	9	4	5	5	6	6	4	4	4	1	4	5	6	2	5	5	3	4	6	4	4	4	100
Herbal flavour^{NS}	12	13	11	8	11	15	9	8	9	13	8	9	10	9	12	8	10	10	7	10	9	11	222

***** Indicates significant differences among samples according to Cochrans Q test at $p \leq 0.001$**

*** Indicates significant differences among samples according to Cochrans Q test at $p \leq 0.05$**

**** Indicates significant differences among samples according to Cochrans Q test at $p \leq 0.01$**

NS Indicates no significant difference among samples according to Cochrans Q test at $p > 0.05$

Significant differences were observed in the frequencies for 25 of the 32 terms in the CATA question, used to describe the various samples.

4.3.2.1. Colour

The term was used mostly for Santa Rossa and R1251. Cucumber, which was a control, had the lowest number of checks. One of the reasons for this may be due to the fact that cucumber peels are naturally a darker shade of green, as opposed to the bright green colour that is synonymous with and desired in nopalitos (Rodríguez & Cantwell, 1988).

4.3.2.2. Appearance

According to Fifield (2018), freshness in all vegetables is important to consumers. Freshness is often characterised by attributes, such as bright lively colours, crispiness and no soft spots on the surface. Cucumber was the sample for which ‘fresh appearance’ was the most frequently used. The lowest scores for ‘fresh appearance’ were for Robusta, Skinners Court and Zastron. For the descriptor ‘thin appearance’, Robusta had the lowest score, because one of the cultivar’s signature traits is that it is heavier and thicker than most of the other nopalito cultivars (Du Toit, 2017). The cucumber scored the highest usage of the “thin appearance” descriptor, mainly due to the thinness of the cucumber slices. Robusta differed significantly from cucumber for the term “thick appearance”.

Cultivars Ofer and Morado had the same number of checks for both “thick appearance” and “thin appearance”, suggesting similarities in appearance. The term ‘slimy appearance’ was minimally used, with the slimiest cultivars being Robusta and Messina. The slimy appearance may be related to the viscosity of the mucilage found in the various cultivars. Du Toit (2017), investigated the viscosity of 42 cladode cultivars using the line-spread, cylinder test and separating funnel. From the results obtained from these three tests as well as the visual physical descriptions of the appearance of the mucilage, it was observed that Robusta and Messina were classified as highly viscous cultivars.

4.3.2.3. Taste

“Sweet taste” was the least used term in this category, to describe any of the samples, with green pepper and cucumber having the most checks, and Malta and Messina having the least. Unsurprisingly, “sweet taste” was the least used term, especially regarding the nopalitos as they are often described as having more of a sharp, sour and tart taste than sweet (Patel, 2014). The term “salty taste” showed a significant difference between Skinners Court and green pepper, and Gymno Carpo and green pepper. Cucumber did not differ in saltiness from Ofer, Robusta, Morado and Rossa. Calvo–Arriaga *et al.* (2010) conducted a study to identify the sensory characteristics that drive the preference of nopalitos in consumers and they found four different nopalito cultivars to have scored a 10 out of 15 value for saltiness with the cultivar

COPENA V1 having recorded as the saltiest (9.08 out of 15). “Bitter taste” had a low frequency of use, with cucumber and green pepper being the least bitter samples, along with cultivar Berg X Mexican. The bitterest sample was Fusicaulis (18). Unsurprisingly, “Bitter taste” had a low frequency of use in this study as all nopalitos were harvested mid-morning where the acid content is the lowest, ensuring less bitter vegetables (Grant, 2020). “Sour taste” was the most frequently used term in this category, with cucumber and green pepper being the least sour samples, while Rossa was the sourest. Sour taste is an important trait as it directly relates to the acidity level of the nopalitos and it is even more important for consumers who prefer nopalitos, which are low in acidity (Rodríguez and Cantwell, 1988). Umami is a distinctive taste that comes as a result of glutamate and 5′-ribonucleotides which are responsible for the acceptability, taste and palatability of food (Yamaguchi & Ninomiya, 2000). The term is Japanese and the closest description of the terms in English includes broth-like, meaty and savoury (Yamaguchi & Ninomiya, 2000). The majority of the samples scored a low frequency of use for the term “savoury/umami taste”. Green pepper and cucumber had the highest number of checks, with Skinners court being the only nopalito cultivar deemed as savoury/umami.

4.3.2.4. Texture

Cucumber was not profiled as being “stalky”, while Robusta and green pepper were perceived as stalky, in that they resembled the texture of vegetables which had edible stems. Chewiness refers to the number of chews which are required to narrow the size of a nopalito to ensure ease of swallowing (Pérez-Cacho *et al.,* 2006). “Chewy texture” was attributed to Zastron, with Robusta and Santa Rossa being not chewy at all. Calvo–Arriaga *et al.* (2010), found chewiness in four nopalito cultivars (Milpa Alta, COPENA F1, COPENA V1 and Tovarito) to be very low with an overall score of 3 out of 15 on the 15 cm linear scale. The least frequently used term in this category was “fibrous texture”. Fibrous texture in this context refers to the nopalitos’ texture containing or resembling fibres (Merriam-Webster.com, 2020). The least fibrous samples were Meyers, Berg X Mexican, Morado and Skinners Court, and the most fibrous sample was Robusta (18). Robusta is one of the thickest cultivars among all nopalito cultivars and thick cultivars have been reported to be stringy (i.e., fibrous) at times (Albert, 2020). The term “slimy texture” was mostly checked for Robusta, with Nudosa being the least slimy cultivar; green pepper and cucumber had almost no sliminess. The slimy texture of nopalitos is attributed to the presence of the substance that is found in the vegetables’ cells known as mucilage which is a thick and slimy liquid that is similar to that found in Aloe Vera (Torres-Acosta, 2007).

“Hard texture” was a descriptor used to evaluate the force that was required to break down the nopalitos against the teeth (Pérez-Cacho *et al*., 2006). Robusta, green pepper and

Fusicaulis were perceived as ‘hard’ in texture, while the least hardness was attributed to Malta, Rossa and Santa Rossa. Morado had a “spongy texture”, with green pepper having no sponginess. Sponginess in this context was attributed to the possibility of the presence of a big proportion of intercellular space inside the nopalitos which would in turn result in a porous texture as observed in Morado (Terefe & Verseeg, 2011). In a study to develop a mayonnaise-like product, Bernardino-Nicanor *et al.* (2015) removed epidermal tissue from the young cladodes of the *Opuntia robusta* Wendl var. robusta and they obtained the parenchymatous tissue which was found to be spongy.

Crispy texture refers to a desirable level of firmness and brittleness, with an ease of crumbliness (Tunick *et al.,* 2013). “Crispy texture” was the most frequently used term to describe the various samples. Green pepper and cucumber were the crispiest, and Robusta was the least crispy, with a check frequency that was 6.25 times lower than that of green pepper. The usage of the term “soft texture” was the lowest for green pepper and the highest for Meyers. Soft in this context, referred to the tenderness that was observed in the samples’ flesh. It was important to investigate this attribute as nopalitos are often regarded as being soft, much of which refers to the flesh of these vegetables (Pérez-Cacho *et al.,* 2006; Teague, 2020)

4.3.2.5. Aftertaste

The term “sweet aftertaste” characterised green pepper and cucumber, while all the nopalito samples had almost no sweet aftertaste, especially Rossa and Malta. “Sour aftertaste” was the most frequently checked term in this category. Turpin had the most checks for sour aftertaste, while green pepper and cucumber were not perceived as having a sour aftertaste. All nopalito samples had a significantly higher usage of this attribute, which differed from the green pepper and cucumber, meaning that all nopalito cultivars exhibited a level of sour aftertaste. The sour aftertaste that was observed in all of the nopalito samples can be attributed to the presence of organic acids that are found in the nopalitos such as acetic acid as well as citric acid (Pérez-Cacho *et al.,* 2006). Cucumber recorded the least frequent usage for the term “bitter aftertaste”, while green pepper was perceived as bitter as Fresno, Ofer and Morado. The sample with the most checks for “bitter aftertaste” was Fusicaulis. The bitterness found in some vegetables can easily be ascribed to the calcium content that is found in these vegetables, in that a high content of this mineral results in increased bitterness (Tordoff & Sandell, 2009). Calcium is one of the minerals that are found abundantly in nopalitos in the form of calcium oxalate deposits (Du Toit, 2017). As such, its presence contributes to the bitterness that was observed. “Savoury aftertaste” was the least frequently used term in this category, with only cucumber having a slight ‘savoury aftertaste’.

4.3.2.6. Flavour

"Grassy flavour" refers to the kind of flavour that is related to fresh-cut grass (Arancibia *et al.,* 2011). This was the most frequently used term in this category. Nopalitos have been deemed to have a unique flavour, and can often be used as an alternative for green vegetables such as green pepper, asparagus and fresh green beans (Orr & Tinsely, 1975). Calvo–Arriaga *et al.* (2010) also reported a "Green flavour" in four nopalito cultivars they studied, which was associated with green raw vegetables such as peas. The samples, scoring the least checks for this term, were green pepper and cucumber. The nopalito cultivar with the grassiest flavour, was Gymno Carpo. "Cucumber flavour" was primarily used to describe cucumber and all the other samples were significantly lower by more than 4.45 times. As with the cucumber, green pepper had the highest usage for the term "green pepper flavour", while all the other samples were also significantly lower for this flavour, by more than 4.45 times. The nopalito cultivar with the highest usage of green pepper flavour was Messina. The term "green bean flavour's" lowest usage was for cucumber and green pepper, while the highest was for Turpin.

Green beans are described to be a little wet, juicy, crunchy and have a slight bitter green flavour (Picky Eater Adventures, 2016). The observation of both "green pepper flavour" and "green bean flavour" in some of the nopalitos of this study further supports previous reports which have suggested an association between all these vegetables in terms of flavour (Rodríguez and Cantwell, 1988; Razo and Sánchez, 2002; Pérez-Cacho *et al.,* 2006; www.perishablenews.com, 2020).

4.3.3. Correspondence Analysis

The first two dimensions of the biplot explained 69.76% of data variability, which was represented by 47.80% for F1 and 21.95% for F2, respectively (Figure 11). Cultivar R1260 was the only nopalito cultivar situated in the right side of the biplot, along with cucumber and green pepper. This cultivar was characterised by descriptors, such as 'fresh appearance', 'fresh flavour', 'no aftertaste' and 'chewy texture'. On the left side of the biplot, all the other nopalito cultivars were clustered together, along with 'negative' descriptors, such as 'slimy appearance', 'sour taste', 'sour aftertaste' and 'bitter taste'.

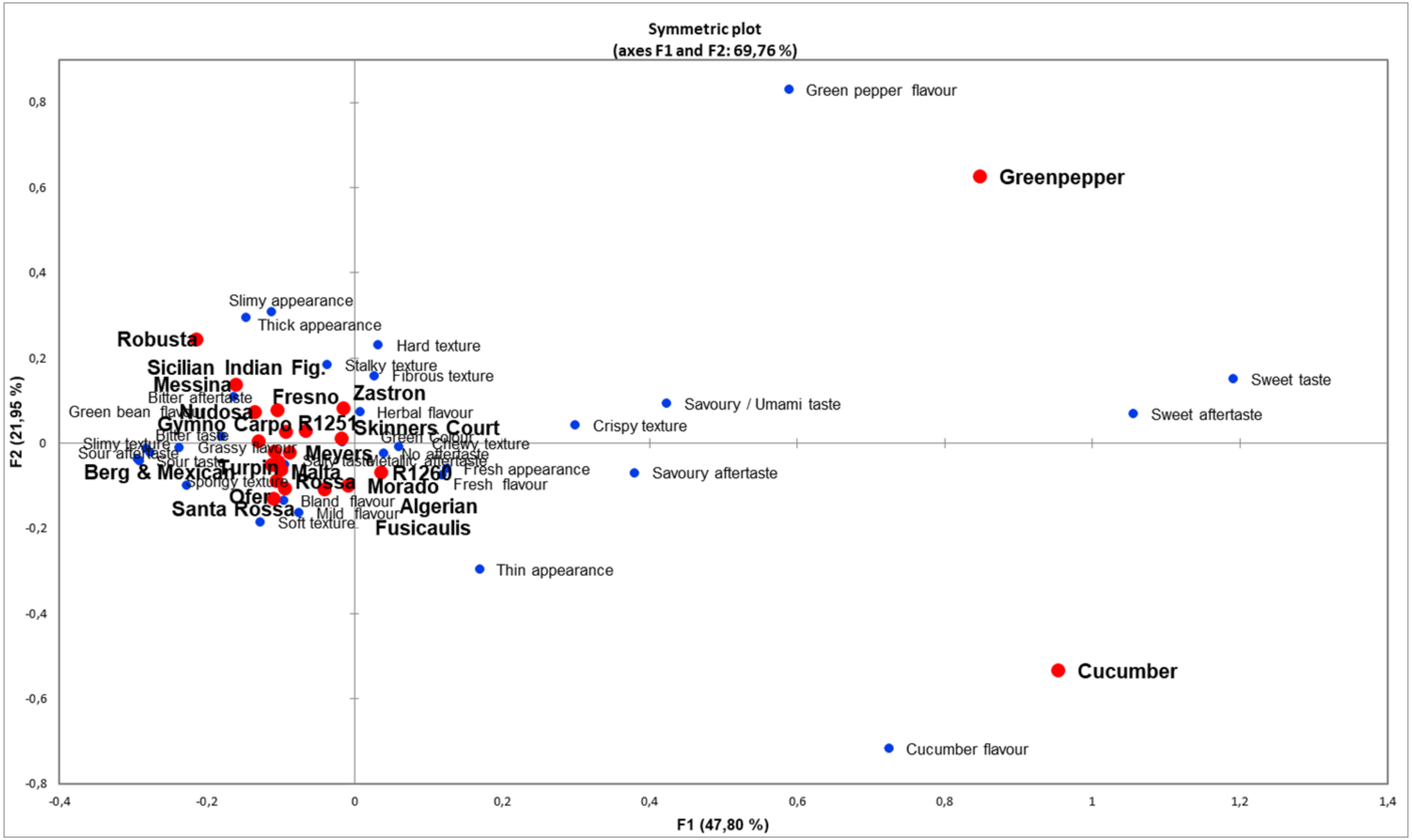

Figure 11: Sample and term representations in the first and second coordinates of the correspondence analysis, performed on the frequency of the CATA questions for the evaluation of nopalitos, from different cactus pear cultivar

Factor 2 divided the biplot into a top and bottom part. Ten of the nopalito cultivars, namely Robusta, Sicillian Indian Fig, Messina, Zastron, Fresno, Nudosa, Gymno Carpo, Turpin, Skinners Court and R125 were grouped, together with green pepper, in the top half of the biplot. The rest of the nopalito cultivars were clustered in the bottom part of the biplot, along with cucumber.

4.3.4 Agglomerative hierarchical clustering and external preference mapping

The dendrogram, presented in Figure 12, comprises various demographic groups, which formed part of the sensory panel (Table 19). Cluster 1, which was the smallest cluster, consisted of 10 participants, which were mostly represented by white (60%) females (80%), in the age groups 18 – 30 years (40%) and 51 – 65 years (40%). Furthermore, it is the cluster with the most equal representation in terms of staff members (50%) and students (50%). Cluster 2 was the biggest (28 participants) and comprised mostly of white (72%) females (75%), who were aged between 18 and 30 (54%). Additionally, this cluster recorded the highest representation of males (25%). Cluster 3 had 11 female participants, including the highest number of Coloured participants (9%) and staff members (64%). Cluster 4 recorded the highest percentage of participants aged between 18 and 30 (75%), as well as the highest number of students (67%). Of these participants, the highest percentage was Africans (67%) and the lowest percentage of participants aged between 51 and 65 (8%).

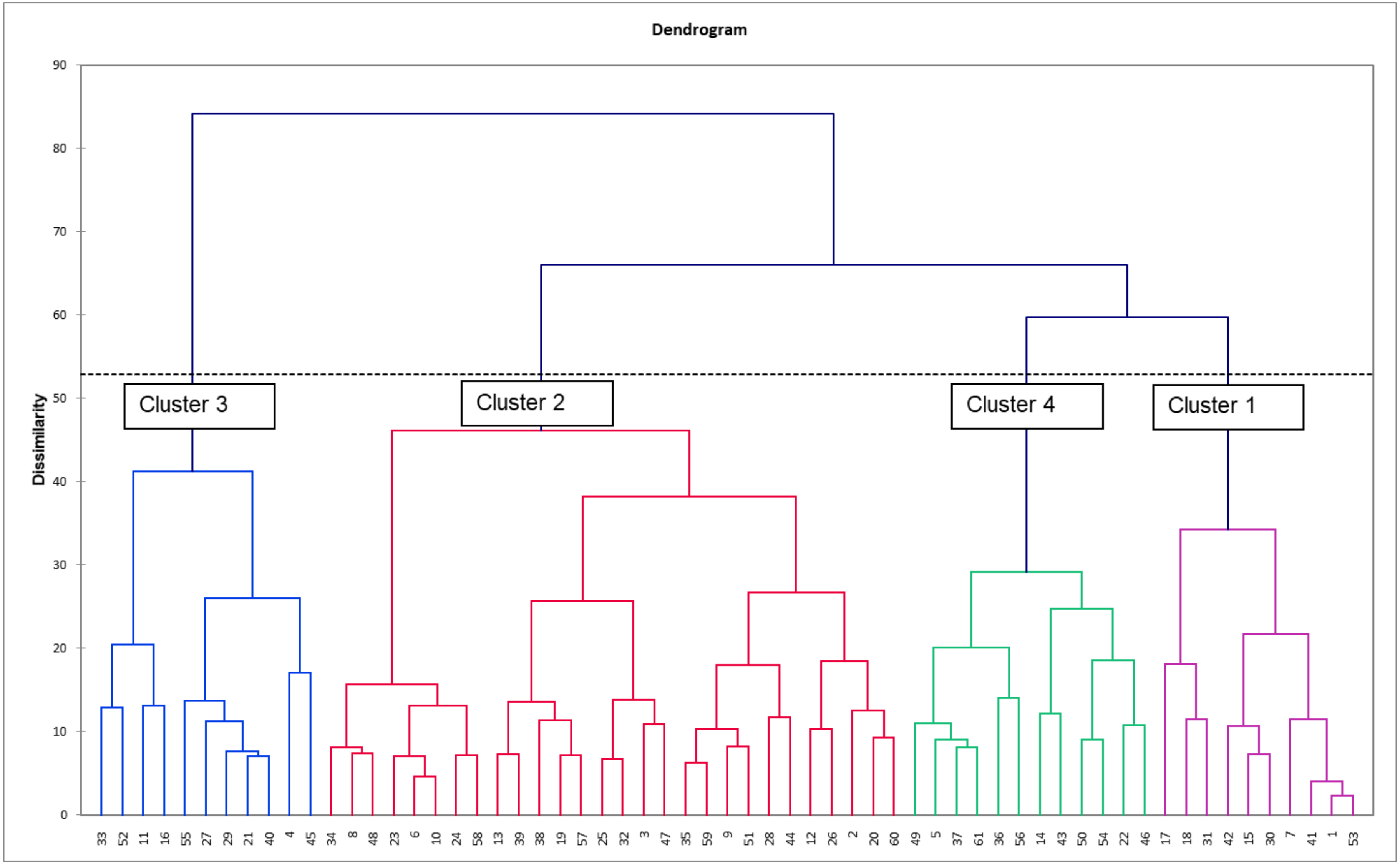

Figure 12: Dendrogram of the AHC, indicating the presence of four clusters of consumer's overall hedonic acceptability of different nopalitos from different cactus pear cultivars

The external preference map (Figure 13) depicts the locations, in which each of the samples were classified, as guided by the consumers' overall likeability for each sample. All clusters (1 – 4) fell in the red region of the plot, which featured cucumber and green pepper. This signifies that 80 – 100% of the consumers gave the highest overall acceptability rankings for these two samples. Furthermore, the plot shows that all 20 nopalito samples were situated in the blue region, meaning that 0 – 20% of the consumers gave the highest rankings to these samples, which correlated with the fact that the majority of the consumers rated the nopalito samples low (4 - 5) on the hedonic scale

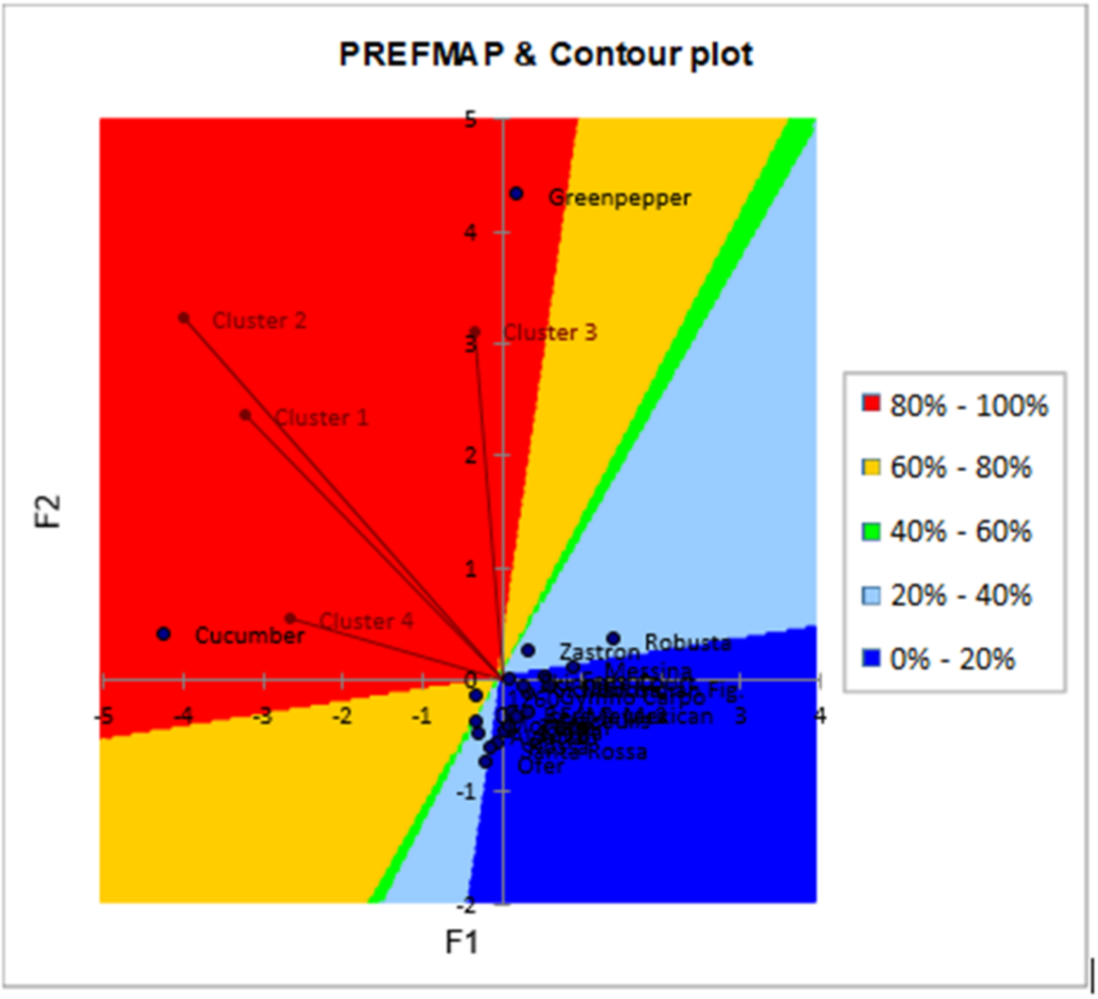

Figure 13: External preference map of identified consumer clusters, of nopalitos from 20 different cultivars, as well as cucumber and green pepper

4.4. Conclusion

Amongst the nopalito cultivars, Skinners Court, had the highest numerical ranking, as it was one of the only four nopalito cultivars which ranked a liking of 5 (neither like nor dislike) on the hedonic scale, while the remaining 16 cultivars ranked an overall liking of 4 (dislike slightly). Furthermore, it was among the four least fibrous nopalito cultivars of the study and it was closely associated with green pepper and cucumber in some of the attributes, such as “salty taste” and “savoury/umami taste”. Robusta was ranked the lowest because it had the thickest and slimiest appearance, as well as the least level of crispness – all attributes which are undesirable in the perfect raw nopalito vegetable. Although certain significant differences were noted between the different nopalito cultivars, it was apparent that the cultivars ranged in similar margins for all attributes and terms, used in this study. It is recommended that future research include product development, which will explore vast arrays of preparation methodology, to increase the overall acceptability, palatability and the masking of undesirable traits of nopalitos for the South African consumer.

Chapter 5

5. The nutritional evaluation of nopalitos from six South African cactus pear cultivars

Abstract

The Opuntia ficus-indica species of the cactus pear plant's use and consumption have advanced over the years because it forms an important part of the human nutritional regime. The high nutrient capacity is ascribed to the species's exceptional content of vitamins, proteins and minerals. The objective of this study was to determine the nutritional properties of the young edible stems (nopalitos). Nutritional analysis was conducted in this appraisal for the six nopalito cultivars which were deemed to be the overall best following the morphological and physico-chemical analyses as well as the consideration of the sensorial properties of the 20 South African cactus pear cultivars. The reported work has shown the high quality of the nopalitos as a vegetable source which can easily and competitively be compared to other known vegetables such as green pepper and lettuce.

5.1. Introduction

The young edible stems of the *Opuntia ficus-indica* species of the cactus pear plant have been consumed and commercialised as vegetables in countries like Mexico and the United States of America for many centuries (Mohamed-Yasseen *et al.,* 1996). The species has since gained prominence as a result of its reported health benefits such as anti-inflammatory (Park *et al.,* 2001), anti-ulcerogenic (Galati *et al.,* 2003), anti-cancer (Zou *et al.,* 2005) and antioxidant (Dehbi *et al.,* 2013) properties. The benefits that come with the consumption of the stems can be ascribed to the vegetable's high fibre content, minerals, vitamins (mainly vitamin A and C) and proteins (Gurrieri *et al.,* 2000). According to Ware (2019), a cup of raw nopalitos contains approximately 141 milligrams (mg) of calcium; 19.8 micrograms (mcg) of vitamin A; 13.8 calories; 8 mg of vitamin C; 4.56 mcg of vitamin K; 2.86 grams (g) of carbohydrate; 1.89 g of fibre; 1.14 g of protein; 0.99 g of sugar and 0.08 g of fat. In one study, the effects of the stems on the treatment of diabetes were tested on 14 patients who were diagnosed with type 2 diabetes (Ware, 2019). The 14 participants were divided into 2 groups, whereby one group was given a carbohydrate-dense breakfast while the other group was given a breakfast which contained nopalitos (López-Romero *et al.,* 2014). The researchers observed that the group which had nopalitos incorporated in their breakfast reported significantly lower insulin and blood sugar levels after their meal in contrast to the group which did not include the stems in their meal. Other studies have shown that the plant can potentially aid in soothing pain, protecting the liver as well as boosting immune activity (Stintzing & Carle, 2005). It is for these reasons, that traditional medicine practitioners have utilised the stems in the treatment and prevention of various conditions such as glaucoma and fatigue (Ware, 2019). Despite its benefits, the vegetable is still almost exclusively used in the countries of its origin. It is as such, imperative to analyse the vegetable's nutritional properties to consider it as a vegetable crop in South Africa. This is all the more important for a country such as South Africa where food security and sustainability are at an all-time low and the majority of the population cannot afford nutritious food. The introduction of nopalitos can be one of the ways in which food insecurity is alleviated as the cactus plant is a low-cost and self-sustaining plant. This study aimed to investigate the nutritional properties found in the young stems of six South African cactus pear cultivars.

5.2. Materials and methods

5.2.1. Sample collection

Six nopalitos from each of the six selected South African cultivars of cactus pear plants were harvested at a length of 12 cm-20 cm one day before the various nutritional analyses took place. The six cultivars are Fresno, Fusicaulis, Malta, Meyers, Morado and Nudosa. The samples were harvested at the Bloemfontein cactus pear research orchard which is found on the West Campus of the University of the Free State. Upon harvesting, the nopalito samples were thoroughly washed under running water, dethorned, rinsed again, and macerated in a food processor (Kenwood Compact 2.1L Food Processor FDP300WH) to form fine, soft crushed nopalitos (pulp). The samples were then placed into separate containers each marked with the name of the cultivars and stored in a cold room at 4°C. All samples were done in duplicate for every experiment.

5.2.2. Crude protein

The protein content of the nopalitos was determined by thermal combustion. The Nitrogen (N) content was determined using a Leco Nitrogen analyser and the crude protein (CP) was automatically calculated by the Leco machine by multiplying the N content by a factor of 6.25 (AOAC, 2000). For the determination of crude protein, 0.6 grams (g) of the fresh macerated nopalito samples were weighed into glass vials and dried overnight (100˚C). Approximately 0.09 g and 0.25 g of the dried matter were placed in foil containers. The correct masses of the samples were then determined, verified and placed in the carousel of the Leco machine for three minutes. The crude protein values were displayed as grams of Crude Protein per kilogram of Dry Matter (g CP/kg DM).

5.2.3. Fibre

Acid Detergent Fibre (ADF), Neutral Detergent Fibre (NDF) and Acid Detergent Lignin (ADL) of the six cultivars of nopalitos were investigated using the methods reported by Goering & Van Soest (1970) and Robertson & Van Soest (1981).

5.2.3.1. Acid detergent fibre

For the investigation of ADF, one gram of each fresh nopalito sample was weighed off and ground. One hundred millilitres (ml) of room temperature acid-detergent solution and 2 ml decahydronaphthalene were then added.

The mixtures were heated for five to ten minutes. In order to avoid foaming, the heat was reduced once the mixtures began to boil. The boiling process was gradually adjusted to a slow, even level over 60 minutes.

The resulting mixtures were then filtered on previously tared Gooch crucibles, which were set on a filter manifold with the aid of light suction. Rods were used to disband the filtered mats which were then washed twice with hot water (90°-100° C). The sides of the crucible were also rinsed in the same manner. To ensure that all the colour was removed, the crucibles were repeatedly washed with acetone and in turn, broke up all lumps so that the solvent came into contact with all particles of fibre (Figure 14).

If the crucibles still contained acetone, hexane was added. The acid-detergent fibre was now free of hexane and dried overnight at 100° C and then weighed.

The acid-detergent fibre was calculated as follows:

$(Wo-Wt)\ (100)/S = ADF$

Where:

Wo = weight of oven-dry crucible including fibre

Wt = tared weight of oven-dry crucible

iS = oven-dry sample weight

Figure 14: Various nopalito samples being investigated for Acid Detergent Fibre content

5.2.3.2. Acid Detergent Lignin

Upon preparing the acid detergent fibre procedure, an amount of asbestos which was equal to the volume of fibre was added to the crucibles to proceed with the test for ADL. The contents of the crucibles were then covered with cooled (15°C) 72% sulphuric acid (H_2SO_4) and stirred with glass rods to obtain a smooth paste. The crucibles were then filled approximately half full with acid and stirred, taking care that the glass rods remained in the crucibles. H_2SO_4 (72%) was refilled into the mixture which was then stirred at hourly intervals as the acid drained away. The crucibles were kept at a constant temperature ranging between 20°C to 23°C for three hours. Once the three hours lapsed, much of the acid was filtered using a vacuum; the contents were then washed with hot water until they were completely free from the acid. The stirring rods were then rinsed off and removed from the crucibles. The crucibles were dried at 100°C and weighed after which they were ignited in a muffle furnace at 500°C to 550°C for three hours, cooled to 100°C and then weighed. The acid-detergent lignin was then calculated as follows:

$(L \times 100)/S = ADL$

Where:

L = loss upon ignition after 72% H_2SO_4 treatment

S = oven-dry sample weight

5.2.3.3. Neutral detergent fibre

Air-dried nopalito samples (0.5 to 1.0 g) which were ground to pass 20 to 30 mesh (1 millimetre) or an equivalent were placed into beakers of the refluxing apparatus. One hundred millimetres of room temperature neutral-detergent solution, 2 ml decahydronaphthalene, and 0.5 g sodium sulfite were added in that order into the aforementioned mixtures with a calibrated scoop. The resulting mixtures were boiled for five to ten minutes, after which the heat was gradually reduced as boiling began, to avoid foaming. The previously tared Gooch crucibles were placed on filter manifolds and filled with the contents of the mixture in the beakers. A low vacuum was used at first and then increased only if more force was required. The samples were then rinsed into crucibles with hot water (90-100°C). The liquid was filtered through a repeated washing procedure. Acetone was used to wash the crucibles twice, dried at 100°C overnight and weighed. The yield of the recovered neutral-detergent fibre was reported as a percent of cell-wall constituents. To estimate cell soluble material, the abovementioned values were subtracted from 100. The residues remaining in the crucibles were ashed for three hours at 500°C to 550°C and weighed off. The ash content was reported as ash insoluble in neutral detergent.

5.2.4. Fat analysis

The method of Folch *et al*. (1957) was utilised to obtain the total lipid content from the nopalito pulp found in the various cultivars (Figure 15). The total lipids were quantitatively extracted, using chloroform and methanol in a ratio of 2:1. Butylated hydroxytoluene, which is an antioxidant was added to the chloroform: methanol mixture at a concentration of 0.001 %. To dry the obtained fat extracts, a rotary evaporator was used under vacuum. These extracts were dried overnight in a vacuum oven at 50°C, using phosphorus pentoxide as a moisture adsorbent. Gravimetric analysis was used to determine total extractable fat and expressed as % fat weight per weight (w/w) per 100 g. The fat-free dry matter (FFDM) content was determined by weighing the residue on a preweighed filter paper which was used for Folch extraction, after drying. The FFDM could be expressed as % FFDM (w/w) per 100 g upon determining the difference in weight of each sample. The moisture content of the seed was determined by subtraction using the formulae: 100% - % lipid - % FFDM. The resulting differences were then expressed as %moisture (w/w) per 100 g. The extracted fat from each sample was then stored in polytops (glass vials with push-in tops) under a blanket of nitrogen and frozen at -20°C pending fatty acid analyses.

Figure 15: Extraction of total lipid content in various nopalito cultivars in progress

5.2.4.1. Fatty acid analysis

A disposable glass pasteur pipette was used to transfer a lipid aliquot of 20 mg into a Teflon-lined screw-top test tube. Fatty acids were then transesterified to ensure the formation of methyl esters using 0.5 N NaOH in methanol and 14 % boron trifluoride (Slover & Lanza, 1979; Hur *et al*., 2004; Diaz *et al*., 2005) (Figure 16). The internal standard which was used in this process was nonadecanoic acid (C19:0).

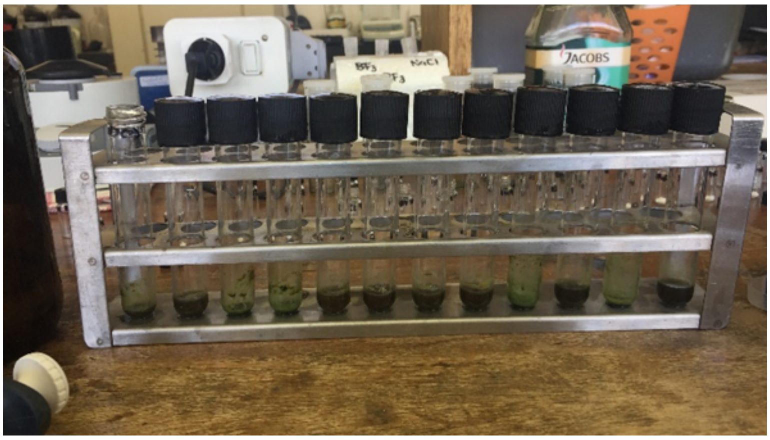

Figure 16: Transesterification of various nopalito cultivars in progress

The Fatty acid methyl esters (FAMEs) from the fat were then quantified using a Varian 430 flame ionisation GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 µm film thickness). Analysis was performed using an initial isothermic period (40°C for 2 minutes). Following that, was an increment of temperature at a rate of 4°C/minute to 230°C. An isothermic period of 230°C for ten minutes followed. A Varian CP 8400 Autosampler was then used to inject 1 µl of the resulting FAMEs n-hexane into columns. The injection port and detector were both conserved at 250°C. Hydrogen, at 45 psi, served as the carrier gas, while nitrogen was utilised as the makeup gas. Galaxy Chromatography Software was employed to record the chromatograms. FAME samples were identified by comparing the retention times of their peaks whose standards were obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). Furthermore, nonadecanoic acid (C19:0) was also obtained from Supelco. The rest of the reagents and solvents which were of analytical grade were obtained from Merck Chemicals (Pty Ltd, Halfway House, Johannesburg, South Africa).

Fatty acids were expressed as the proportion of each individual fatty acid to the total of all fatty acids present in each sample. The following fatty acid combinations were then calculated: omega-3 (*n*-3) fatty acids; omega-6 (*n*-6) fatty acids; total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA/SFA ratio (P/S) and *n*-6/*n*-3 ratio.

5.2.5. Mineral analysis

Cactus pear plants are known to imply a great source of minerals. The calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) and phosphorous (P) were determined using the dry-ashing procedure 968.08 (AOAC, 2000). The minerals' ion concentrations were then measured with a double-beam atomic absorption spectrometer (Analyst 300, Perkin Elmer, USA) and expressed as grams per kilogram of dry matter (g/kg DM).

5.2.6. Sucrose/D-Glucose/D-Fructose analysis

The UV-method kit (Boehringer-Mannheim, 1999) was used to determine sucrose, D-glucose as well as D-fructose found in the nopalito samples. The Sucrose/D-Glucose/D-Fructose kit (Sucrose / D-Glucose / D-Fructose Boehringer Mannheim / R-Biopharm Enzymatic BioAnalysis / Food Analysis) which was used involved the following steps (Figure 17):

5.2.6.1. Blank sucrose samples

Twenty microlitres (µl) of solution 1 were pipetted into cuvettes and these were left to incubate for five minutes at 37°C. Following the incubation, 100 µl of solution 2 was added to the cuvettes together with 180µl of distilled water. The resulting solution was left to stand for three minutes upon which the first absorbance reading (A1) was taken. To start the reaction, 2 µl of suspension 3 was added to the cuvettes, after which were left to react for 15 minutes. Once the reaction had stopped, the second absorbance reading (A2) was taken.

5.2.6.2. Sucrose samples

Twenty microlitres of solution 1 were pipetted into cuvettes together with 10 µl of each nopalito sample and these were left to incubate for five minutes at 37°C. Following the incubation, 100 µl of solution 2 was added to the cuvettes together with 170 µl of distilled water. The resulting solution was left to stand for three minutes upon which the first absorbance reading (A1) was taken. To start the reaction, 2 µl of suspension 3 was added to the cuvettes and left to react for 15 minutes. Once the reaction had stopped, the second absorbance reading (A2) was taken.

5.2.6.3. Blank D-glucose/D-fructose samples

One hundred microlitres of solution 2 were added to the cuvettes together with 200 µl of distilled water. The resulting solution was left to stand for three minutes upon which the first absorbance reading (A1) was taken. To start the reaction, 2 µl of suspension 3 was added to the cuvettes, after which were left to react for 15 minutes. Once the reaction had stopped, the second absorbance reading (A2) was taken. The last step involved an addition of 2 µl of suspension 4 to the samples and the absorbance (A3) was read after 15 minutes.

5.2.6.4. D-glucose/D-fructose samples

Ten microlitres of sample solution were added to the cuvettes and these were left to incubate for five minutes at 37°C. Following the incubation, 100 µl of solution 2 was added to the cuvettes together with 190 µl of distilled water. The resulting solution was left to stand for three minutes upon which the first absorbance reading (A1) was taken. To start the reaction, 2 µl of suspension 3 was added to the cuvettes and left to react for 15 minutes. Once the reaction had stopped, the second absorbance reading (A2) was taken. The last step involved an addition of 2 µl of suspension four to the samples and the absorbance (A3) was read after 15 minutes. Following the conduction of the four abovementioned experiments, the differences in absorbance were determined to identify the different sugars found in the samples. Determining the aforementioned, involved the following calculations:

$$\Delta A = (A2 - A1)sample - (A2 - A1)blank$$

The difference between $\Delta AtotalD - glucose\ (from\ sucrose\ sample)\ and\ \Delta AD - glucose\ (from\ D - glucose\ sample)$ yielded $\Delta ASucrose$

D-fructose was determined as follows:

The absorbance differences $(A3 - A2)$ for both the blank and samples $(D - glucose/D - fructose)$ were obtained after which, the blank absorbance differences were subtracted from that of the samples. This thus resulted in $\Delta AD - fructose$.

The concentrations of sucrose, D-glucose and D-fructose were then calculated as follows:

$c = \frac{V\ x\ MW}{\varepsilon \text{ x d x v x } 1000} = \Delta A\ [g/L]$

Where:

V = final volume [mL]

MW = molecular weight of the substance assayed [g/mol]

ε = extinction coefficient of NADPH at 340 nm = 6300 [l x mol^{-1} x cm^{-1}]

d = light path [cm]

v = sample volume [mL]

The concentrations for sucrose were determined as follows:

$c = \frac{3.020\ x\ 342.3}{\varepsilon \text{ x } 1.00 \text{x } 0.100 \text{ x } 1000}\ x\ \Delta Asucrose = \frac{10.34}{\varepsilon}\ x\ \Delta Asucrose\ [g\ sucrose/l\ sample\ solution]$

The concentrations for D-glucose were determined as follows:

$c = \frac{3.020\ x\ 180.16}{\varepsilon \text{ x } 1.00 \text{x } 0.100 \text{ x } 1000}\ x\ \Delta Asucrose = \frac{5.441}{\varepsilon}\ x\ \Delta Asucrose\ [g\ sucrose/l\ sample\ solution]$

The concentrations for D-fructose were determined as follows:

$c = \frac{3.040\ x\ 180.16}{\varepsilon \text{ x } 1.00 \text{x } 0.100 \text{ x } 1000}\ x\ \Delta Asucrose = \frac{5.477}{\varepsilon}\ x\ \Delta Asucrose\ [g\ sucrose/l\ sample\ solution]$

The final values were then converted to mg/g.

5.2.7. Starch analysis

The UV-method kit (Boehringer-Mannheim, 1999) was used to determine the native starch found in the nopalito samples. A starch kit (Boehringer Mannheim / R-Biopharm Enzymatic BioAnalysis / Food Analysis) was used and the experiment was conducted as follows (Figure 17):

5.2.7.1. Reagent blank

Twenty microlitres of solution 1 were pipetted into cuvettes together with 10 µl of distilled water and these were left to incubate for 15 minutes at 55°C. Following the incubation, 100 µl of solution 2 was added to the cuvettes together with 100 µl of distilled water. The resulting solution was left to stand for three minutes upon which the first absorbance reading (A1) was taken. To start the reaction, 2 µl of suspension 3 was added to the cuvettes and left to react for ten minutes. Once the reaction had stopped, the second absorbance reading (A2) was taken.

5.2.7.2. Sample

Twenty microlitres of solution 1 were pipetted into cuvettes together with 10 µl of the sample solution and these were left to incubate for 15 minutes at 55°C. Following the incubation, 100 µl of solution 2 was added to the cuvettes together with 100 µl of distilled water. The resulting solution was left to stand for three minutes upon which the first absorbance reading (A1) was taken. To start the reaction, 2 µl of suspension 3 was added to the cuvettes and left to react for ten minutes. Once the reaction had stopped, the second absorbance reading (A2) was taken.

5.2.7.3. Sample blank

Ten microlitres of sample solution were pipetted into cuvettes and these were left to incubate for 15 minutes at 55°C. Following the incubation, 100 µl of solution 2 was added to the cuvettes together with 120 µl of distilled water. The resulting solution was left to stand for three minutes upon which the first absorbance reading (A1) was taken. To start the reaction, 2µl of suspension 3 was added to the cuvettes and left to react for ten minutes. Once the reaction had stopped, the second absorbance reading (A2) was taken. Following the conduction of the abovementioned experiments, the differences in absorbance were determined in order to quantify the starch found in the samples.

Determining the aforementioned, involved the following calculations:

$$\Delta A = (A2 - A1)sample - (A2 - A1)reagent\ blank$$

The concentrations of starch in the various nopalito samples were then calculated as follows:

$$c = \frac{V\ x\ MW}{\varepsilon\ x\ d\ x\ v\ x\ 1000} = \Delta A\ [g/L]$$

Where:

V = final volume [mL]

MW = molecular weight of the substance assayed [g/mol] (for starch: $MW_{D\text{-glucose}} - MW_{water}$ = 162.1)

ε = extinction coefficient of NADPH at 340 nm = 6300 [$l \times mol^{-1} \times cm^{-1}$]

d = light path [cm]

v = sample volume [mL]

The final values were then converted to g/100g.

Figure 17: Preparation of samples for sugar and starch analyses

5.2.8. Phytochemicals analysis

Cactus pear plants are known to be a great source of phytochemicals (Guevara-Figueroa *et al.,* 2010) and the following were investigated in the various nopalito samples:

5.2.8.1. Polyphenol analysis

Total phenolic compounds were determined using two grams of watery extracts from the nopalito samples which were obtained through a 15-minute centrifugation step at 8000 revolutions per minute (rpm) speed. These were then incorporated with 1 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate solution concentration and then left to react for 30 minutes. Absorbance values of the samples were read at 765 nanometers (nm) using a Genesys 10 Vis Thermos Spectronic spectrophotometer. The readings were expressed as milligrams of gallic acid equivalents per litre (mg/GAE), subsequently, after a calibration curve was obtained using gallic acid at a concentration of 0, 50, 100, 150, 200, 250, 300 and 350 milligrams per litre (mg/l) (Luximon-Ramma *et al*., 2002).

5.2.8.2. Chlorophyll analysis

Zero point five grams of each nopalito sample was accurately weighed off and homogenised with 10 ml of diethyl-ether (DEE) which served as the extractant solvent. The resulting homogenised samples were then centrifuged at a speed of 10 000 rpm and a temperature of 4°C for 15 minutes to obtain the supernatants of the samples. The supernatants were put asunder from the sediment and 0.5 ml of the former were combined with 4.5 ml of corresponding solvents namely, 80% acetone and diethyl-ether (DEE). Chlorophyll-a (Ch-a), Chlorophyll-b (Ch-b), as well as the total carotenoids (Cx+c) found in the resulting mixtures, were then determined. To calculate the aforementioned variables using the DEE solvent, the following equations were used (Lichtentaler & Wellburn, 1985):

$$\text{Ch-a} = 10.05A_{663nm} – 0.97A_{663nm}$$

$$\text{Ch-b} = 16.36A_{645nm} – 2.43A_{645nm}$$

$$Cx+c = (1000A_{470nm} – 1.43C_a – 35.872C_b)/205$$

5.2.8.3. Flavonoid analysis

The total flavonoid content in the nopalito samples was determined by using the colourimetric method (trichloride aluminium method) adopted by Bahorun *et al.* (1996). An aliquot of methanol extracts (0.3 ml) was added to a 5 ml volumetric flask, containing 0.45 ml of distilled deionised water. Once five minutes lapsed, 0.75 ml of 2% aluminium chloride ($AlCl_3.H_2O$) solution was added. The mixture was shaken and allowed to rest for ten minutes to allow the reaction to take place. The absorbance was measured at 415 nm against the prepared methanol blank with a UV-VIS spectrophotometer (Spectrometer Spectra Max 384 PLUS Molecular Devices). The resulting values were expressed as milligrams of quercetin equivalent per gram (mg QE/g).

5.2.8.4. Ascorbic acid (Vitamin C) analysis

The 2,6 dichlorophenol indophenol (DCPIP) titration method was used to determine the ascorbic acid content found in the various nopalito samples (James, 1995). A solution of 0.04% of 2,6 DCPIP solution was used to titrate 10-fold extracts from the nopalito samples. The samples were titrated until a faint pink colour (titration volume T) was achieved. The experiment was recapitulated with 5 ml of distilled water which was used as a blank (titration volume B) and 5 ml of a 0,02% solution of ascorbic acid (titration volume St.) was used as a standard. The resulting ascorbic acid values were then expressed as mg/100g fresh weight using the equation below:

$$\frac{mg}{100\text{mg}} = \frac{(T-B)}{(St\ -B)(20)(DF)}$$

Where:

T = titration value

B = blank

St = standard solution (5.18)

DF = dilution factor

The resulting values were converted to mg/100g.

5.2.9. Antioxidant activity analysis

5.2.9.1. FRAP (Ferric Reducing Antioxidant Power) analysis

The FRAP assay administered by Pérez-Jimenez *et al*. (2008) was used in this experiment. Nine hundred microlitres of the FRAP reagent were combined with 90 µl of distilled water as well as 30 µl of nopalito samples. Absorbance values were taken at 595 nm after every 15 seconds at 37°C. The readings that were observed at 30 minutes were selected for calculating the FRAP value of each sample. Estimates of antioxidant capacity were determined using a standard curve and the resulting values were expressed as micromole ascorbic acid equivalent (AAE) per gram (µmol AAE/g).

5.2.9.2. ORAC (Oxygen Radical Absorbance Capacity)

The ORAC assay procedure was conducted with references from the work reported by Ou *et al.* (2001). The experiment was conducted at 37 °C under pH 7.4 using Trolox which is a water-soluble analogue of vitamin E as a control standard.

The resulting measurements were expressed as micromole Trolox equivalents (TE) per gram (µmol TE/g).

5.2.10. Statistical analyses

The effect of the cultivar on all nutritional components was analysed by analysis of variance (ANOVA) (NCSS 11 Statistical Software 2016). The means were compared with the Tukey-Kramer multiple comparison test at $\alpha = 0.05$ (NCSS 11 Statistical Software 2016).

5.3. Results and discussion

The results for the various nutritive parameters which were evaluated in the six nopalito cultivars on a dry matter basis are summarised in Tables 22 and 23. In these tables, the existence of significant differences can be observed ($p < 0.05$) among the averages of the majority of the samples and parameters which will all be discussed below.

Table 22: Analysis of variance (ANOVA) for the effect of cultivar on the various nutritional properties of nopalitos

Cultivar	Fresno	Fusicaulis	Malta	Meyers	Morado	Nudosa	Average	Sign. level
% Moisture	89.96^{a} ± 0.22	90.74^{b} ± 0.25	89.84^{a} ± 0.15	90.75^{b} ± 0.44	92.06^{c} ± 0.13	91.25^{b} ± 0.19	90.77	$p < 0.001$
Crude protein (g/kg DM)	93.29^{a} ± 0.38	101.28^{b} ± 0.64	129.62^{f} ± 2.11	124.60^{e}± 2.37	119.12^{d} ± 0.27	114.18^{c} ± 0.83	113.68	$p < 0.001$
% Fat	0.61^{b} ± 0.02	0.55^{a} ± 0.04	0.71^{c} ± 0.01	0.61^{b} ± 0.03	0.59^{ab} ± 0.02	0.59^{ab} ± 0.01	0.61	$p < 0.001$
% FFDM	9.43^{c} ± 0.23	8.71^{b} ± 0.25	9.44^{c} ± 0.14	8.65^{b} ± 0.44	7.35^{a} ± 0.11	8.16^{b} ± 0.19	8.62	$p < 0.001$
Neutral-detergent fibre (g/kg DM)	241.81^{b} ± 5.55	177.54^{a} ± 1.76	180.62^{a} ± 6.09	239.37^{b} ± 17.80	234.26^{b} ± 28.41	230.06^{b} ± 16.37	217.28	$p < 0.001$
Acid-detergent fibre (g/kg DM)	87.60^{b} ± 1.47	85.94^{ab} ± 1.32	78.76^{a} ± 0.72	92.22^{bc} ± 2.01	90.61^{bc} ± 5.67	97.11^{c} ± 5.17	88.71	$p < 0.001$
Acid-detergent lignin (g/kg DM)	16.32^{a} ± 1.54	35.71^{c} ± 0.11	24.71^{b} ± 1.58	41.23^{d} ± 0.71	25.38^{b} ± 0.41	39.17^{d} ± 0.94	30.42	$p < 0.001$
Glucose (mg/g)	2.67^{ab} ± 0.39	2.87^{ab} ± 0.34	3.58^{bc} ± 0.52	2.38^{a} ± 0.06	2.33^{a} ± 0.06	4.09^{c} ± 0.70	2.99	$p < 0.001$
Starch (g/100g)	2.93^{c} ± 0.74	0.21^{a} ± 0.25	1.57^{b} ± 0.17	0.21^{a} ± 0.25	0.01^{a} ± 0.01	0.57^{a} ± 0.66	1.7	$p < 0.001$
Ca (g/kg DM)	37.43^{d} ± 0.02	28.29^{ab} ± 0.18	30.80^{bc} ± 0.24	29.98^{abc} ± 3.54	32.50^{c} ± 0.09	27.30^{a} ± 1.02	31.05	$p < 0.001$
Mg (g/kg DM)	15.15^{d} ± 0.52	14.23^{cd} ± 0.55	12.55^{b} ± 0.64	10.37^{a} ± 0.68	13.71^{bc} ± 0.40	10.76^{a} ± 0.41	12.80	$p < 0.001$
Na (g/kg DM)	0.31^{ab} ± 0.10	0.43^{bc} ± 0.10	0.55^{c} ± 0.10	0.36^{ab} ± 0.04	0.37^{abc} ± 0.02	0.24^{a} ± 0.10	0.38	$p < 0.001$
K (g/kg DM)	22.96^{bc} ± 1.62	25.91^{c} ± 3.50	12.48^{a} ± 0.84	14.07^{a} ± 2.50	15.16^{a} ± 2.36	21.08^{b} ± 0.09	18.61	$p < 0.001$
P (g/kg DM)	2.24^{a} ± 0.37	2.43^{a} ± 0.15	2.61^{ab} ± 0.19	2.60^{ab} ± 0.01	3.34^{c} ± 0.14	2.98^{bc} ± 0.21	2.70	$p < 0.001$
Vitamin C (mg/100g)	136.82^{b} ± 8.95	141.67^{b} ± 7.83	132.95^{ab} ± 2.24	124.22^{ab} ± 3.36	131.01^{ab} ± 11.19	115.50^{a} ± 8.95	130.36	$p < 0.001$
Flavonoids (mg QE/g)	0.20 ± 0.02	0.20 ± 0.06	0.23 ± 0.02	0.30 ± 0.03	0.26 ± 0.10	0.23 ± 0.02	0.24	$p = 0.101$
Polyphenols (mg GAE/g)	1.92^{a} ± 0.06	1.90^{a} ± 0.06	3.05^{c} ± 0.10	3.17^{c} ± 0.08	3.03^{c} ± 0.04	2.21^{b} ± 0.03	2.55	$p < 0.001$

Carotenoids (DEE mg/g)	0.45^{ab} ± 0.04	0.80^{b} ± 0.05	0.06^{a} ± 0.07	2.99^{c} ± 0.02	0.33^{a} ± 0.14	0.40^{ab} ± 0.46	0.84	$p < 0.001$
Chlorophyll a (DEE mg/g)	2.69^{b} ± 0.41	2.00^{ab} ± 0.57	2.68^{b} ± 0.53	6.40^{c} ± 0.18	2.10^{ab} ± 0.31	1.53^{a} ± 0.14	2.90	$p = 0.002$
Chlorophyll b (DEE mg/g)	1.24^{b} ± 0.07	0.90^{ab} ± 0.89	0.73^{ab} ± 0.63	0.01^{a} ± 0.01	1.53^{b} ± 0.06	5.44^{c} ± 0.01	1.64	$p = 0.003$
FRAP (µmol AAE/g)	1.46^{a} ± 0.09	1.70^{abc} ± 0.09	1.79^{bcd} ± 0.12	2.01^{d} ± 0.16	1.88^{cd} ± 0.14	1.55^{ab} ± 0.15	1.73	$p < 0.001$
ORAC (µmol TE/g)	83.34^{a} ± 4.55	75.51^{a} ± 1.49	99.97^{c} ± 8.56	99.09^{c} ± 3.29	94.52^{bc} ± 3.02	84.04^{ab} ± 3.88	89.41	$p < 0.001$

Means with different superscripts in the same row differ significantly

5.3.1. Crude protein

Crude protein measures the amount of protein in food which is key to good health, immune function, oxygen transport and maintaining strong muscle tissue (Tremblay, 2019). A high content of protein is usually associated with high-quality nopalitos. The cultivars all differed significantly ($p < 0.001$) from each other with the average being 113.68 g/kg DM (Table 22). Malta yielded the highest crude protein content (129.62 g/kg DM), while Fresno yielded the lowest content (93.29 g/kg DM). When comparing these results to other cultivars that have been reported by various researchers, they seem to be higher than the latter. For example, the six cultivars in this appraisal are higher in crude protein than five out of the six cultivars of the *Opuntias Mill. spp* that were presented by Mohamed-Yasseen *et al.* (1996). These previously reported cultivars which were obtained from various parts of the world are *O. amyclaea* Engelm. from Italy (40 g/kg DM); *O. brasiliensis* Willd. from Australia (65 g/kg DM); *O. chollo* Welo. from the USA (55 g/kg DM); *O. maxima* Mill. from South Africa (65 g/kg DM) and O. *ficus-indica (L.)* Mill. from Italy (96 g/kg DM).

5.3.2. Fibre

Nopalitos are celebrated for their high fibre content which plays a role in reducing body weight by binding to dietary fat, thus reducing the dietary fat which is available for absorption (Hernández-Urbiola *et al.,* 2010). The NDF (Neutral Detergent Fibre) analysis observes the plant cell's structural components such as cellulose and hemicellulose (Van Saun, 2013). Furthermore, it quantifies how much food consumers can hold. Carpenter (2019) has reported that there is a limit to how much food consumers can consume at once. For example, a ruminant will consume food up until such a point that the rumen is full (Carpenter, 2019). The ruminant will no longer eat once the rumen is full until the food is digested or moves to the gut (Carpenter, 2019). A high NDF value signifies the reduced ability and potential of a consumer to eat plant material due to the high presence of the plant cell wall material (Van Saun, 2013). The significance of the high presence of these cell wall components, in turn, implies that the fibre found in the food will take up space in the stomach, resulting in less ability of the food to provide large amounts of energy (Carpenter, 2019). The six nopalito cultivars averaged an NDF content of 217.28 g/kg DM with Fresno recording the highest content (241.81 g/kg DM) while Fusicaulis scored the lowest NDF content (177.54 g/kg DM) (Table 22). No significant differences were observed between Nudosa (230.06 g/kg DM), Morado (234.26 g/g DM) and Meyers (239.37 g/kg DM). However, Fusicaulis and Malta (180.62 g/kg DM) yielded significantly ($p < 0.001$) lower NDF contents than the four other cultivars. This means that Fusicaulis and Malta would be better cultivars than the four aforementioned cultivars in terms of consumers being able to consume them as they contain less plant cell wall material and would provide more energy for these consumers.

Acid Detergent Fibre (ADF) measures the indigestible plant material, excluding hemicellulose (Novotny *et al.,* 2017). A low value signifies high digestibility and nutrient availability which is more desired than a high value. This means that as the ADF concentration increases in plants, the concentration of digestible energy decreases (Novotny *et al.,* 2017). The ADF content among the six nopalito cultivars ranged between 78 g/kg DM and 98 g/kg DM with an average of 88.71 g/kg DM (Table 22). Significant ($p < 0.001$) differences were observed between the cultivars except for differences between Morado (90.61 g/kg DM) and Meyers (92.22 g/kg DM). Malta had a significantly ($p < 0.001$) lower (78.76 g/kg DM) ADF content as compared to Nudosa, Fresno, Morado, Fresno and Fusicaulis. On the other side of the spectrum, Nudosa recorded a significantly ($p < 0.001$) higher ADF content (97.11 g/kg DM) as compared to Meyers, Fresno, Fusicaulis and Morado. Furthermore, Malta, Fusicaulis (85.94 g/kg DM) and Fresno (87.60 g/kg DM) were all significantly ($p < 0.001$) lower than the other three cultivars as well as lower than the average of the six cultivars. These results, in turn, mean that Malta is more digestible and more nutritive than Nudosa.

Acid Detergent Lignin (ADL) is used to measure the lignin fraction of ADF. Low values of ADL are most desirable as they signify a softer nopalito. Furthermore, the presence of high amounts of lignin decreases the digestibility of plant cell wall material, the intake of the plant material by consumers as well as the performance of these consumers after consumption of the plant material (Chaves *et al.,* 2002). The six cultivars yielded an average of 30.42 g/kg DM (Table 22). Meyers yielded a significantly ($p < 0.001$) high ADL content (41.23 g/kg DM) as compared to the five cultivars, while Fresno recorded the lowest ADL content (16.32 g/kg DM). The difference between the lowest ADL-containing cultivar (Fresno) and the highest ADL-containing cultivar (Meyers) was significantly high as Meyers was higher than Fresno by 2.5 fold. No significant differences were observed between Malta (24.71 g/kg DM) and Morado (25.38 g/kg DM), however, they recorded values that were lower than the average of the six cultivars, just as with Fresno. Fusicaulis (35.71 g/kg DM), Nudosa (39.17 g/kg DM) and Meyers (41.23 g/kg DM) yielded significantly higher ADL values than Fresno, Morado and Malta. This means that these three aforementioned cultivars would be the least ideal to provide consumers with maximum energy to be able to perform daily tasks upon consumption.

In comparison to ADF and NDF, the content of ADL in all nopalito cultivars was significantly lower. A possible explanation for this is that nopalitos are harvested while still young and the concentration of lignin only increases in the cell wall as the plant matures, which often leads to a tough, stringy texture. Rodrigues *et al.* (2016) investigated six *Opuntia ficus-indica* cladodes from Portuguese ecotypes for their ADL, NDF and ADF contents. On average, those six cultivars (PT1ecotype, PT2ecotype, PT3ecotype, PT4ecotype, PT5ecotype cv and “Gialla”) scored lower values in terms of the NDF (185.15 g/kg DM), as well as the ADL (8.85

g/kg DM) content while they scored a higher value in terms of the ADF (107.04 g/kg DM) content as compared to the results obtained in this appraisal.

Table 23: The fatty acid composition of the six nopalito cultivars

Cultivar		**Fresno**	**Fusicaulis**	**Malta**	**Meyers**	**Morado**	**Nudosa**	**Average**	**Sign. level**
FAME (% of total fatty acids):									
Common name:	Abbreviation:								
Myristic	C14:0	0.40^{b} ± 0.10	0.37^{ab} ± 0.04	0.26^{a} ± 0.05	0.28^{ab} ± 0.03	0.28^{ab} ± 0.03	0.29^{ab} ± 0.05	0.31	$p = 0.012$
Palmitic	C16:0	31.07^{c} ± 0.67	33.54^{d} ± 0.47	28.71^{a} ± 0.45	30.51^{bc} ± 0.16	29.65^{ab} ± 0.25	32.81^{d} ± 0.83	31.05	$p < 0.001$
Palmitoleic	C16:1c9	0.86 ± 0.15	0.88 ± 0.14	0.97 ± 0.09	0.86 ± 0.33	0.94 ± 0.05	0.95 ± 0.28	0.91	$p = 0.934$
Margaric	C17:0	0.46^{ab} ± 0.04	0.42^{a} ± 0.05	0.46^{ab} ± 0.04	0.42^{a} ± 0.02	0.52^{b} ± 0.03	0.52^{b} ± 0.05	0.54	$p = 0.004$
Heptadecenoic	C17:1c10	1.53^{c} ± 0.04	1.86^{d} ± 0.10	0.75^{a} ± 0.21	1.19^{b} ± 0.16	0.81^{a} ± 0.09	1.12^{b} ± 0.12	1.21	$p < 0.001$
Stearic acid	C18:0	6.36^{c} ± 0.12	6.93^{d} ± 0.19	4.96^{a} ± 0.22	5.87^{b} ± 0.14	6.01^{bc} ± 0.19	6.20^{bc} ± 0.30	6.06	$p < 0.001$
Oleic	C18:1c9	6.76^{a} ± 0.40	7.51^{ab} ± 0.30	10.99^{d} ± 0.37	11.11^{d} ± 0.34	9.72^{c} ± 0.45	7.93^{b} ± 0.20	10.62	$p < 0.001$
Vaccenic	C18:1c7	1.03^{ab} ± 0.06	1.04^{ab} ± 0.08	1.13^{b} ± 0.02	1.10^{ab} ± 0.02	0.98^{a} ± 0.06	1.30^{c} ± 0.07	1.10	$p < 0.001$
Linolelaidic	C18:2t9,12 (n-6)	3.63^{abc} ± 0.08	3.92^{abc} ± 0.13	3.44^{ab} ± 0.35	3.34^{a} ± 0.10	4.06^{c} ± 0.18	3.98^{bc} ± 0.50	3.73	$p = 0.005$
Linoleic	C18:2c9,12 (n-6)	26.68^{b} ± 0.37	24.41^{a} ± 0.48	26.76^{b} ± 0.95	26.50^{b} ± 0.70	26.72^{b} ± 0.34	24.72^{a} ± 0.35	25.97	$p < 0.001$
Arachidic	C20:0	0.54 ± 0.05	0.58 ± 0.03	0.51 ± 0.11	0.54 ± 0.05	0.43 ± 0.07	0.48 ± 0.04	0.51	$p = 0.056$
α-Linolenic	C18:3c9,12,15 (n-3)	19.92^{cd} ± 0.75	17.64^{ab} ± 0.52	20.31^{d} ± 0.39	17.16^{a} ± 0.36	18.91^{bcd} ± 0.37	18.77^{bc} ± 1.18	18.79	$p < 0.001$
Eicosatrienoic	C20:3c8,11,14 (n-6)	0.46 ± 0.07	0.56 ± 0.05	0.56 ± 0.16	0.63 ± 0.06	0.53 ± 0.11	0.57 ± 0.08	0.55	$p =0.324$
Lignoceric	C24:0	0.30^{ab} ± 0.06	0.27^{a} ± 0.03	0.39^{abc} ± 0.07	0.48^{c} ± 0.04	0.42^{bc} ± 0.09	0.35^{abc} ± 0.06	0.36	$p = 0.003$
Fatty acid ratios:									
Total Saturated Fatty Acids (SFA)		39.14^{cd} ± 0.80	42.16^{e} ± 0.59	35.29^{a} ± 0.73	38.10^{bc} ± 0.20	37.31^{b} ± 0.29	40.66^{de} ± 1.07	38.78	$p < 0.001$

Total Mono Unsaturated Fatty Acids (MUFA)	10.17^{a} ± 0.56	11.30^{b} ± 0.37	13.64^{d} ± 0.27	14.26^{d} ± 0.79	12.46^{c} ± 0.42	11.30^{b} ± 0.25		$p < 0.001$
Total Poly Unsaturated Fatty Acids (PUFA)	50.69^{b} ± 1.08	46.54^{a} ± 0.87	51.07^{b} ± 0.46	47.63^{a} ± 0.94	50.23^{b} ± 0.19	48.04^{a} ± 1.08	49.03	$p < 0.001$
Total Omega- 6 Fatty Acids (n-6)	30.77^{b} ± 0.34	28.89^{a} ± 0.47	30.75^{b} ± 0.85	30.47^{b} ± 0.66	31.32^{b} ± 0.38	29.27^{a} ± 0.31	30.25	$p < 0.001$
Total Omega- 3 Fatty Acids (n-3)	19.92^{cd} ± 0.75	17.64^{ab} ± 0.52	20.31^{d} ± 0.39	17.16^{a} ± 0.36	18.91^{bcd} ± 0.37	18.77^{bc} ± 1.18	18.79	$p < 0.001$
PUFA:SFA	1.30^{cd} ± 0.05	1.10^{a} ± 0.04	1.45^{e} ± 0.04	1.25^{bc} ± 0.03	1.35^{d} ± 0.01	1.18^{ab} ± 0.06	1.27	$p < 0.001$
n-6:n3	1.55^{ab} ± 0.04	1.64^{abc} ± 0.04	1.51^{a} ± 0.07	1.78^{c} ± 0.03	1.66^{bc} ± 0.05	1.56^{ab} ± 0.10	1.62	$p < 0.001$

Means with different superscripts in the same row differ significantly

5.3.3. Fat analysis

5.3.3.1. Total fat, moisture content and total fat-free dry matter (FFDM) content

Nopalitos are regarded as low-calorie vegetables as a result of their low fat and high water content (Stintzing & Carle, 2005). When observing the resulting total percentage fat and moisture contents of the various cultivars, it became evident that the results support the aforementioned statement, as all values of fat were below 1% while the moisture content was close to 100% (Table 22). The total percentage fat content of the six nopalito cultivars averaged 0.61% with Malta yielding a significantly ($p < 0.001$) higher fat content (0.71%) than the five cultivars, while Fusicaulis yielded significantly ($p < 0.001$) the lowest total fat content (0.55%) as compared to Fresno, Morado, Malta, Meyers and Nudosa. No significant differences were observed between Morado (0.59%) and Nudosa (0.59%) nor were there any significant differences observed between Meyers (0.61%) and Fresno. All of the values that were observed were below 1%, which is highly consequential as it further confirms the low-calorie nature of the crop and coincides with results that have been reported by various researchers. For example, the USDA National Nutrient Database (2018) has shown that the fat content of raw nopalitos amounted to 0.09 g per 100 g, which is also less than 1%. This supports what was observed in this study.

The moisture content of the cultivars ranged between 89% and 92.5% with an average moisture content of 90.77% (Table 22). Morado presented a significantly ($p < 0.001$) high moisture content (92.06%) as compared to the five nopalito cultivars, while Malta recorded the lowest (89.84%) moisture content. No significant differences were observed between Fusicaulis (90.74%), Meyers (90.75%) and Nudosa (91.25%). Furthermore, no significant differences were observed between Malta and Fresno (89.96%); however, they were lower than the average moisture content. All cultivars recorded high moisture contents which makes them beneficial for human consumption as high moisture may assist in weight regulation and maintenance. Furthermore, it quantifies nopalitos as being an efficient source of forage for animals, especially in long periods of drought.

Du Toit (2017) has reported that the level at which a food product is moist cannot solely be determined from the product’s moisture content as several other components contribute towards the overall moistness, such as the fat content of that product. It has further been reported that the fat content of the product also contributes to its textural properties (Du Toit, 2017). As such, it becomes important to investigate the portion of the food product that is devoid of fats as well as water to quantify other nutrients that are present in the food product (Du Toit, 2017). This portion is known as the FFDM. The average %FFDM in this study was 8.62% (Table 22). Malta recorded a significantly ($p < 0.001$) high (9.44%) %FFDM as compared to all the cultivars in the study, while Morado showed to contain significantly (p <

0.001) the lowest (7.35%) %FFDM as compared to the five remaining cultivars. No significant differences were observed between Nudosa (8.16%), Meyers (8.65%) as well as Fusicaulis (8.71%). The FFDM values of Morado and Nudosa were lower than the average of the cultivars. Furthermore, no significant differences were observed between Fresno (9.43%) and Malta (9.44%).

5.3.3.2. Fatty acid analysis

Fatty acids are long-chained hydrocarbons that are classified into four categories namely: saturated, monounsaturated, polyunsaturated, as well as trans fats (White, 2009). White (2009) has further reported that there are more than twenty kinds of fatty acids that are found in food, all of which exhibit several health benefits. The analyses of total lipids extracted from the nopalitos showed that palmitic acid (C16:0), linoleic acid (C18:2), α-linolenic acid (C18:3c9,12,15 (n-3)), oleic acid (C18:1c9), stearic acid (C18:0) and linolelaidic acid (C18:2t9,12 (n-6)) contributed significantly to the total fatty acid content (Table 23). It must be noted that although the concentrations of the six aforementioned fatty acids were high, each fatty acid found was a fraction of the overall total % fat, which was essentially lower than 1%. The average content of the fatty acids that were found in extremely low concentrations i.e., were present in less than 1% of the total fatty acids, included palmitoleic (0.91%), eicosatrienoic (0.55%), margaric (0.54%), arachidic (0.51%), lignoceric (0.36%) and myristic acid (0.31%). The average contents of heptadecenoic (1.21%) and vaccenic (1.10%) both peaked slightly above the 1% of total fatty acids. Margaric acid ($p = 0.004$), heptadecenoic acid ($p < 0.001$), vaccenic acid ($p < 0.001$) and lignoceric acid ($p < 0.001$) are the only relatively low fatty acids that were observed to have significant differences in this study. Margaric acid, which is also known as heptadecanoic acid, is an odd-chained (C_{17}) saturated fatty acid (Sheldrick, 2015). This particular fatty acid has been reported to potentially reverse the early stages of diabetes (Sheldrick, 2015). Sheldrick (2015), reported on a study that was conducted on the fatty acid blood levels in forty-nine dolphins. Of the fifty-five fatty acids that were studied, margaric acid seemed to have had the most beneficial impact on metabolism. The six nopalito cultivars that were investigated in this study, revealed interesting observations as some of them reported equal amounts of margaric acid (Table 23). Fusicaulis and Meyers reported 0.42% of margaric acid; Fresno and Malta reported 0.46%, while Morado and Nudosa reported 0.52% of margaric acid. These results as well as the report by Sheldrick (2015) are highly consequential because it has been reported that several investigators in the early 1900s denied the existence of this odd-chained fatty acid for several years on end (Shriner *et al.,* 1933).

Heptadecenoic acid is a monounsaturated fatty acid which has been reported as a potential marker of microbial biomass (Vlaeminck *et al.*, 2005). In this study, Malta (0.75%) and Morado

(0.81%) were significantly ($p < 0.001$) lower than all of the nopalito cultivars in terms of heptadecenoic acid content, as they were below the 1% mark of total fatty acids, while the rest of the cultivars were above this mark (Table 23). Albeit, they were above the 1% mark, Nudosa (1.12%) and Meyers (1.19%) were lower than the average heptadecenoic acid content found in all of the cultivars. Fusicaulis (1.86%) and Fresno (1.53%) were significantly different from one another and recorded significantly higher contents of heptadecenoic acid than all of the cultivars of the study. It was interesting to observe that the results obtained in this study were considerably higher in heptadecenoic acid than those reported by Du Toit (2017) on freeze-dried mucilage. For one, the Morado cultivar reported 0.81% of heptadecenoic acid in this study while it showed a lower value of 0.05% in the freeze-dried mucilage.

Vaccenic acid is a geometric and positional isomer of oleic acid (Lock *et al*., 2004) which has been reported to alter the risks of cancer and coronary heart disease (Field *et al.,* 2009). In this study, Morado (0.98%) was significantly ($p < 0.001$) lower than the five cultivars (Table 23). Furthermore, it recorded a lower content of vaccenic acid than the average of the cultivars. No significant differences were observed in Fresno (1.03%), Fusicaulis (1.04%) and Meyers (1.10%). Nudosa (1.30%) and Malta (1.13%) were significantly ($p < 0.001$) higher than the rest of the cultivars. The results obtained are highly paramount, especially Morado which, although slightly lower than all cultivars, is easily comparable to the amount of vaccenic acid that is found in milk. Precht and Molkentin (1996) have reported that this acid constitutes 1.7% of the total fatty acid content of milk fat.

Lignoceric acid is a 24-carbon chain saturated fatty acid. In this study, no significant differences were observed between Nudosa (0.35%) and Malta (0.39%) (Table 23). Fusicaulis (0.27%) and Fresno (0.30%) were significantly ($p = 0.003$) lower than the rest of the cultivars as well as the average of the cultivars while Meyers (0.48%) and Morado (0.42%) were significantly ($p = 0.003$) higher. It was interesting to observe that the results obtained in this study were all higher in lignoceric acid than the results that were reported by Du Toit (2017) on freeze-dried mucilage. For one, the Morado cultivar showed 0.42% in this study while it showed a significantly lower value of 0.04% in the freeze-dried mucilage. These findings further cement the fact that the same nutrients may vary in amounts in the various parts of cactus plants. No significant differences were observed between the different cultivars in terms of the following acids: myristic, palmitoleic, arachidic and eicosatrienoic acid.

The six main fatty acids (palmitic acid, linoleic acid, α-linolenic acid, oleic acid, stearic acid and linolelaidic acid) which were found in abundance in the nopalitos will be discussed extensively below. These fatty acids contributed an average of 31.05% (palmitic acid), 25.97% (linoleic acid), 18.79% (α-linolenic acid), 10.62% (oleic acid), 6.06% (stearic acid) and 3.73%

(linolelaidic acid), respectively. In this manner, these six individual fatty acids represented over 90% of the total fatty acids which were found in the nopalitos with palmitic acid, linoleic and linolenic acids being the major fatty acids found. Several researchers have indicated that cacti, particularly the fruits, pulp, seeds as well as peels were rich in linolenic, oleic and palmitic acids (Ramadan and Mörsel, 2003a, 2003b, 2003c; Ennouri *et al.,* 2009) and from the results obtained in this study, one can say that the same applies for the stems. Furthermore, Abidi *et al.* (2009) investigated the various fatty acids found in cladodes which showed linoleic acid (34.87%), linolenic acid (32.83%), palmitic acid (13.87%) and oleic acid (11.16%) to have been the most abundant in cladodes, which further support the results obtained in this study. When the researchers compared the linoleic acid content of the cladodes with that of various crops, it was revealed that the stems contained a closely related content of the fatty acid with argan oil (29% to 41%) but were however lower than the content that was found in soybeans (53.30%) and barley (51.26%) (Charouf and Guillaume, 2007; Abidi *et al.,* 2009a).

Palmitic acid is a 16-carbon chain saturated fatty acid, which is the most common saturated fatty acid (Carta *et al.,* 2017). This fatty acid can be found naturally in the human body or can be supplemented through diet or synthesised internally from other fatty acids, amino acids and carbohydrates (Carta *et al.,* 2017). In this study, the fatty acid ranged between 28.71% (Malta) and 33.54% (Fusicaulis) (Table 23). The overall average of this fatty acid between the cultivars was 31.05%. Significant ($p < 0.001$) differences were observed between the different cultivars. Malta and Morado (29.65%) were lower than the average of the cultivars. No significant differences were observed between Nudosa (32.81%) and Fusicaulis, however, they were significantly ($p <0.001$) higher than all the nopalito cultivars. When comparing these values to the average palmitic acid values that were found in cactus freeze-dried mucilage powders (14.07%) and seed oils (15.39%) which were reported by Du Toit (2017) and De Wit *et al.* (2016) respectively, one can deduce that the values found in this study were higher than those that were reported previously. This means that nopalitos contain higher concentrations of this fatty acid than the other parts of the plant.

Linoleic acid is an 18-carbon chain polyunsaturated fatty acid which is essential for developmental and growth purposes at 1 to 2% of daily energy (Hansen *et al.*, 1958). With the ever-increasing agricultural shift towards the use of plant oils which are high in this acid such as corn and soybeans over the years, the daily intake of the fatty acid has increased from 1-2% to over 7% (Blasbalg *et al.,* 2011; Micha *et al.,* 2014). Linoleic acid averaged 25.97% in this study. No significant differences were observed between Meyers (26.50%), Fresno (26.68%), Morado (26.72%) and Malta (26.76%) (Table 23). Furthermore, no significant differences were observed between Fusicaulis (24.41%) and Nudosa (24.72%), however, these two cultivars were significantly ($p < 0.001$) lower than the four aforementioned cultivars as well as the average of the cultivars for this fatty acid. When comparing these values to the average linoleic acid values that were found in cactus freeze-dried mucilage powders (62.14%) and seed oils (61.42%) which were reported by Du Toit (2017) and De Wit *et al.* (2016) respectively, one can deduce that the values found in this study were significantly lower than those that were reported previously. This means that nopalitos contain lower concentrations of this fatty acid in comparison to other parts of the plant.

Alpha (α-) linolenic acid is an 18-carbon chain polyunsaturated omega-3 fatty acid which exhibits anti-inflammatory, antidepressant and neuroprotective properties (Blondeau *et al.,* 2015). This fatty acid is only derived from plants, as such, it can only be derived through dietary intake (Blondeau *et al.,* 2015). The average of this fatty acid among the nopalito cultivars was 18.79% (Table 23). Significant ($p < 0.001$) differences were observed between the cultivars. Meyers (17.16%), Fusicaulis (17.64%)), Morado (18.91%) and Nudosa (18.77%) were not significantly different from each other but were lower ($p < 0.001$) than Fresno (19.92%) and Malta (20.31%). Malta showed the highest value (20.31%). Whitbread (2020) found the following levels of α-linolenic acids in plant foods: flaxseed (53,368%), chia seeds (17,830%), hemp seeds (8,684%), canola oil (9,137%), soybean oil (6,789%), edamame (0,358%), navy beans (0,177%), avocados (0,111%), whole wheat bread (0,137%) and oatmeal (0,018%). When comparing the α-linolenic acid content of the foods observed by Whitbread (2020) with those obtained in this study, one can deduce that only flaxseed oil was substantially higher than the nopalito cultivars. Chia seeds contained a higher α-linolenic acid content than Meyers and Fusicaulis. Albeit, some of the foods that were reported by Whitbread (2020) may have a higher fat content, the observation brings insight into the fact that nopalitos may be an excellent choice for consumers to supplement their daily α-linolenic acid intake, since this fatty acid can only be derived from plants.

Oleic acid is an 18-carbon chain monounsaturated omega-9 fatty acid that is required in human nutrition as well as for the reduction of low-density lipoprotein cholesterol (LDL), total cholesterol and glycemic index levels (Rubio *et al*., 2009). Examples of foods in which this fatty acid is predominantly found include avocados and olive oil (Gunnars, 2017).

The average of this fatty acid among the nopalito cultivars was 10.62% (Table 23). Meyers (11.11%) and Malta (10.99%) were significantly ($p < 0.001$) higher than all cultivars. Fresno (6.76%) was significantly ($p < 0.001$) low together with Fusicaulis (7.51%), Nudosa (7.93%) and Morado (9.72%) as compared to the rest of the cultivars. When comparing these values to the average oleic acid values that were found in cactus freeze-dried mucilage powders (19.12%) and seed oils (18.77%) which were reported by Du Toit (2017) and De Wit *et al.* (2016) respectively, one can deduce that the values found in this study were significantly lower than those that were reported previously. This means that nopalitos contain lower concentrations of this fatty acid in comparison to other parts of the plant.

Stearic acid is an 18-carbon chain saturated fatty acid of which the major sources are meat, poultry, fish, grain products as well as milk and milk products (Kris-Etherton *et al.,* 2005). Furthermore, fats which have been reported to be rich in this fatty acid include beef tallow, butter, cocoa butter (usually consumed as chocolate), mutton tallow and lard (Kris-Etherton *et al*., 2005; U.S. Department of Agriculture, 2007). The stearic acid average among the nopalito cultivars of this study was 6.06% (Table 23). Significant differences were observed among the cultivars ($p < 0.001$). Fusicaulis (6.93%) and Fresno (6.36%) recorded stearic acid contents which were significantly ($p < 0.001$) higher than the remaining four cultivars, while Malta (4.96%) and Meyers (5.87%) contents were significantly ($p < 0.001$) lower than Morado, Fusicaulis, Fresno and Nudosa. No significant differences in stearic acid content were observed between Morado (6.01%) and Nudosa (6.20%). When comparing these values to the average stearic acid values that were found in cactus freeze-dried mucilage powders (2.95%) and seed oils (2.95%) which were reported by Du Toit (2017) and De Wit *et al*. (2016) respectively, one can deduce that the values found in this study were significantly higher than those that were reported previously. This means that nopalitos contain higher concentrations of this fatty acid than the other parts of the plant.

Linolelaidic acid is an 18-carbon chain omega-6 polyunsaturated fatty acid which is an isomer of linoleic acid or conjugated linoleic acid with an ability to modulate immune function, obesity and atherosclerosis (The Human Metabolome Database, 2020). The average of this fatty acid among the nopalito cultivars was 3.73% (Table 23). Significant ($p = 0.005$) differences were observed among the cultivars. Morado (4.06%) and Nudosa (3.98%) were significantly ($p = 0.005$) higher than all other cultivars. Meyers (3.34%) was the cultivar which recorded the lowest linolelaidic acid content among the six cultivars. This cultivar was also lower than the average linolelaidic acid content of the six cultivars, together with Malta (3.44%) and Fresno (3.63%). No significant differences were observed between Fresno and Fusicaulis (3.92%).

5.3.3.3. Fatty acid ratios

The highest ratio of fatty acids that were found in the different nopalito cultivars is polyunsaturated fatty acids (PUFA) followed by saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). These fatty acids averaged 49.03%, 38.78% and 12.19%, respectively (Table 23). In this regard, PUFA were significantly higher than SFA and MUFA. The presence of PUFA in higher quantities than the two other fatty acid ratios is significant as they are known for lowering low-density lipoprotein in the body. These results further cement Wolfram *et al*.'s (2002) reports of nopalitos' cholesterol-lowering properties.

Polyunsaturated fatty acids contain multiple carbon double bonds in their structures and include fatty acids which have C18:2 and C18:3 bonds. These fatty acids are found in animal and plant foods, such as salmon, vegetable oils, some nuts and seeds (Wax, 2018). Moderate consumption of polyunsaturated fatty acids is beneficial for human health as they aid in lowering the risks of heart disease (Van De Walle, 2018). In this study, the average of total polyunsaturated fatty acids was 49.03% (Table 23). Malta was the cultivar which recorded a significantly ($p < 0.001$) higher total PUFA content (51.7%) as compared to the five remaining cultivars while Fusicaulis recorded the lowest content (46.54%). Fresno (50.69%) and Morado (50.23%) values were higher than the average of the six cultivars together with Malta, while Meyers (47.63%) and Nudosa (48.04%) were lower than the average together with Fusicaulis. No significant differences were observed between Malta, Fresno and Morado, nor were they observed between Fusicaulis, Meyers and Nudosa. Du Toit (2017) investigated the total PUFA content of freeze-dried mucilage powders while De Wit *et al.* (2016) investigated the total PUFA content in seed oils, both from the *Opuntia* species of cactus plants. The average total PUFA content of freeze-dried mucilage powders was 62.51% while the seed oils recorded an average of 61.68%. When comparing those results with the ones observed in this study, one can deduce that the freeze-dried mucilage powders as well as the seed oils, contained a decidedly higher PUFA content.

Omega-3 and Omega-6 are two major classes of polyunsaturated fatty acids (Van De Walle, 2018). They are regarded as essential fatty acids which are required for brain function as well as cell growth, however, cannot be synthesised by the body and, as such are obtained through diet (Di Pasquale, 2009). Omega-3 fats are unsaturated fats which play a role in the development of the brain, eyes as well as nerves in infants while they assist in maintaining immune health and lowering risks of heart disease in adults (UnlockFood.ca, 2018). Examples of omega-3 fats which are found include α-linolenic acid, docosahexaenoic acid and eicosapentaenoic acid (UnlockFood.ca, 2018). Only α-linolenic acid was found in high levels in this study. Excellent sources of these fats include fatty fish such as salmon, flaxseeds, walnuts and tofu (UnlockFood.ca, 2018). The total omega-3 fatty acids among the nopalito cultivars had an average of 18.79% (Table 23). Significant ($p < 0.001$) differences were observed between the different cultivars. Malta (20.31%) was observed to contain significantly ($p < 0.001$) the highest total omega-3 fatty acid content as compared to all of the cultivars in the study while Meyers (17.16%) had the lowest content. Apart from Meyers, which was the cultivar that recorded the lowest content of total omega-3 fatty acids, low contents of this fatty acid were also observed in Fusicaulis (17.64%) and Nudosa (18.77%). Morado (18.91%) and Fresno (19.92%) on the other hand, had higher contents of omega-3 fatty acids than the average of the six cultivars in addition to Malta, which had the highest content.

Omega-6 fats are unsaturated fats which play a role in the regulation of genes as well as the promotion of blood clotting and immune health (UnlockFood.ca, 2018). Omega-6 fats which are found include linoleic acid, arachidonic acid, gamma-linolenic acid, as well as linolelaidic acid (UnlockFood.ca, 2018). Excellent sources of these fatty acids include soybeans, safflower, nuts and seeds as well as meat, poultry and fish (UnlockFood.ca, 2018). The total omega-6 fatty acids among the nopalito cultivars had an average of 30.25% (Table 23). Morado (31.32%) was significantly ($p < 0.001$) higher in omega-6 fatty acid content than all cultivars, while Fusicaulis (28.89%) had the lowest content (significantly). No significant differences were observed between Meyers (30.47%), Malta (30.75%), Fresno (30.77%) and Morado. Fusciaulis and Nudosa were not significantly different from each other.

Simopoulos (2002) reported that several studies suggested that human beings have evolved over the years on a diet which primarily consisted of an omega-6 to omega-3 ratio (n-6:n-3) of approximately 1. An increased omega-6/omega-3 ratio has been found to have adverse reactions which may result in the promotion of pathogenesis of diseases such as autoimmune diseases, cancer and cardiovascular disease (Simopoulos, 2002). On the other side of the spectrum, low omega-6/omega-3 ratios have been shown to suppress negative effects (Simopoulos, 2002). In one study which sought to investigate the secondary inhibition of cardiovascular disease, it was observed that a ratio of 4:1 was associated with a decrease in total mortality by up to 70% (Simopoulos, 2002). The average of the n-6:n-3 ratio among the nopalito cultivars was 1.62%. Significant ($p < 0.001$) differences were observed between the cultivars (Table 23). Meyers (1.78%) was significantly ($p < 0.001$) higher than all cultivars in terms of the n-6:n-3 ratios observed while Malta (1.51%) was significantly ($p < 0.001$) lower than all cultivars. Fresno (1.55%) and Nudosa (1.56%) were lower than the average of the cultivars together with Malta, while Fusicaulis (1.64%) and Morado (1.66%) were higher than the average of the cultivars. These values imply a low omega-6:omega-3 ratio, which validates that nopalitos exhibit exceptional nutrients which have several health benefits. As with the n-6:n-3 ratios, the PUFA:SFA ratio values are best when low, as a high PUFA:SFA ratio in diets enhances oxidative stress due to PUFA's high susceptibility to lipid peroxidation (Kang *et al.,* 2005). The PUFA:SFA average among the nopalitos of this study was 1.27% (Table 2). Significant differences ($p < 0.001$) were observed in this study. Malta (1.45%) and Morado (1.35%) were significantly higher than all other cultivars. Fusicaulis (1.10%) was significantly low. Fusicaulis was lower than the average of the cultivars together with Nudosa (1.18%) and Meyers (1.25%), while Fresno (1.30%) was higher than the average together with Malta (1.45%) and Morado (1.35%).

Saturated fats are usually regarded as unhealthy and the cause of heart disease as they are often grouped with tans-fats which are known to cause health problems (Kubala, 2020). It must, however, be noted that saturated fatty acids from plant sources are good for one's health. These saturated fatty acids which may be beneficial to good health are those that contain sixteen (C16:0) or eighteen (C18:0) carbon chains. Saturated fatty acids do not contain any double bonds in their carbon chains and are important in human nutrition as they can elevate blood lipid levels (Aukema & Campbell, 2011). Such fatty acids include those that contain C16:0 and C18:0 chains. Excellent sources which are concentrated with these fatty acids and may come with health benefits include coconut products such as coconut oil and unsweetened coconut flakes as well as grass-fed whole milk yoghurt and grass-fed meat (Kubala, 2020). It has been reported that an intake of coconut oil has been shown to improve high-density lipoprotein (HDL) cholesterol and weight loss while the consumption of full-fat dairy intake has shown neutral or protective effects on heart disease risks (Drouin-Chartier *et al.,* 2016; Wallace, 2019). The average saturated fatty acid content of the cultivars in this study amounted to 38.78% (Table 23). Fusicaulis (41.16%) showed the highest total SFA content (statistically significant $p < 0.001$ as compared to Fresno, Malta, Meyers, Morado and Nudosa), while Malta recorded the lowest content (35.29%), compared to the other five cultivars. Morado (37.31%) and Meyers (38.10%) were lower than the average of the cultivars together with Malta, while Fresno (39.14%) and Nudosa (40.66%) were higher than the average together with Fusicaulis. Du Toit (2017) and De Wit *et al*. (2016) reported on the presence of total SFA content of freeze-dried mucilage powders and seed oils from *Opuntia* cactus pear plants. The total SFA content from these two studies averaged 17.51% and 18.70%, respectively. This proves that the different parts of the cactus pear plant can contain varying amounts of the same component.

Monounsaturated fatty acids only contain one double bond in their structure. Diets which are rich in this kind of fatty acids have been shown to have favourable cardiovascular and anti-inflammatory benefits as they allow for improved lipid profiles (Cheah *et al.,* 2019). The average of the total MUFA content among the cultivars in this study was 12.19% (Table 23). Fresno (significantly $p < 0.001$) recorded the lowest total MUFA content as compared to the rest of the cultivars (10.17%), while Meyers was significantly ($p < 0.001$) higher (14.26%) than all of the cultivars in the study. No significant differences were observed between Nudosa (11.30%) and Fusicaulis (11.30%); however, these two cultivars were lower than the average (12.19%) of the cultivars. The total MUFA content that was found in Meyers and Malta (13.64%) was not significantly different to one another, although these two cultivars were higher than the average of the nopalitos together with Morado (12.46%).

5.3.4. Mineral analysis

Minerals are elements on earth and in foods that are required for normal body function and development (National Institutes of Health, 2019). The minerals which are required for bodily function are called essential minerals which can further be categorised into macrominerals and microminerals (Healthwise, 2019). The two categories are equally beneficial for bodily function, however, microminerals are required in small amounts (Healthwise, 2019). Examples of macrominerals include sodium, calcium, phosphorous, magnesium and potassium, while microminerals include iron, iodine, zinc, fluoride and magnesium (Healthwise, 2019). The minerals which were found in abundance in the nopalitos of this study were all macro namely, calcium (Ca), potassium (K), magnesium (Mg), phosphorous (P) and sodium (Na), respectively.

Calcium which is highly regarded for its ability to improve bone strength in humans (Healthwise, 2019) and is essential for membrane function in plants, averaged 31.05 g/kg DM in this study (Table 22). Significant ($p < 0.001$) differences were observed among the nopalito cultivars. The mineral was found to be significantly ($p < 0.001$) higher in Fresno (37.43 g/kg DM) as compared to Nudosa, Fusicaulis, Malta, Meyers and Morado. Nudosa recorded a significantly ($p < 0.001$) lower content (27.30 g/kg DM) as compared to the remaining cultivars. Nudosa, Fusicaulis (28.29 g/kg DM), Meyers (29.98 g/kg DM) and Malta (30.80 g/kg DM) were all below the average of the cultivars while Morado (32.50 g/kg DM) and Fresno were higher than the average (31.05 g/kg) of the six cultivars.

Potassium which is an essential mineral in maintaining the electrolyte balance in plant cells and the human body (Healthwise, 2019) averaged 18.61 g/kg DM among the nopalito cultivars of this study (Table 22). Significant ($p < 0.001$) differences were observed among the nopalito cultivars. The mineral was found to be significantly ($p < 0.001$) higher in Fusicaulis (25.91 g/kg DM) as compared to Fresno, Malta, Morado, Meyers and Fresno. Malta (12.48 g/kg DM) on the other side of the spectrum yielded the lowest potassium content. No significant differences were observed between Malta, Meyers (14.07 g/kg DM) and Morado (15.16 g/kg DM), all of which were significantly lower than Fusicaulis, Fresno and Nudosa. These three cultivars were further lower than the average of the cultivars. Nudosa (21.08 g/kg DM) and Fresno (22.96 g/kg DM) were also significantly higher than the three aforementioned cultivars and were higher than the average (18.61 g/kg) as with Fusicaulis.

Magnesium which is required for the body to make proteins (Healthwise, 2019) averaged 12.80 g/kg DM (Table 22). Significant ($p < 0.001$) differences were observed among the cultivars. Fresno (15.15 g/kgDM) was significantly ($p < 0.001$) higher than all cultivars while Meyers (10.37 g/kg DM) was significantly lower than Fresno, Fusicaulis, Malta, Morado and

Nudosa. No significant differences were observed between Meyers and Nudosa (10.76 g/kg DM), however, both of these cultivars recorded a magnesium content which was lower than the average of the cultivars in addition to Malta (12.55 g/kg DM). Apart from Fresno, other cultivars which recorded a magnesium content that was higher than the average (12.80 g/kg) were Fusicaulis (14.23 g/kg DM) and Morado (13.71 g/kg DM).

Phosphorous which is essential for healthy teeth and bones (Healthwise, 2019) averaged 2.70 g/kg in this study (Table 22). Significant ($p < 0.001$) differences were observed among the cultivars. Morado (3.34 g/kg DM) yielded a significantly ($p < 0.001$) higher phosphorous content as compared to all cultivars while Fresno (2.24 g/kg DM) yielded the lowest phosphorous content. No significant differences were observed between Fresno and Fusicaulis (2.43 g/kg DM). No further significant differences were observed between Meyers (2.60 g/kg DM) and Malta (2.61 g/kg DM). Morado and Nudosa (2.98 g/kg DM) were higher than the average (2.70 g/kg) of the cultivars.

Sodium which plays a role in maintaining the electrolyte balance in the body (Healthwise, 2019) averaged 0.38 g/kg DM (Table 22). Significant ($p < 0.001$) differences were observed among the cultivars. This mineral was found to be significantly ($p < 0.001$) higher in Malta (0.55g/kg DM) as compared to Fresno, Fusicaulis, Meyers, Morado and Nudosa, while it was significantly ($p < 0.001$) lower in Nudosa (0.24 g/kg DM) as compared to all the cultivars of this study. No significant differences were observed in the sodium content found in Fresno (0.31 g/kg DM) and Meyers (0.36 g/kg DM), however, both of these cultivars were below the average sodium content of all cultivars together with Morado (0.37 g/kg DM). The highest contents of this mineral were found in Fusicaulis (0.43 g/kg DM) and Malta, which were the only cultivars whose content was higher than the average of all the cultivars. One of the most interesting observations that the results revealed was that the calcium content of all cultivars was higher than the potassium content which supports Bicalho and Penteado's (1981) observation which found that approximately 60% of the mineral content of young cladodes was calcium. This is different from Stintzing and Carle's (2005) observation which showed potassium to be the main mineral in nopalitos, amounting to approximately 60%. While magnesium (Mg) was undetected in a study reported by Muñoz de Chávez *et al.* (1995), the mineral was seemingly traced as the third-highest mineral (12.80 g/kg DM) found in the nopalito cultivars of this study, surpassing Sodium which was reported as the third most abundant mineral found in cladodes by Stintzing and Carle (2005). Another mineral which was observed in higher quantities than sodium in this study is phosphorus (P) which averaged 2.70 g/kg DM.

5.3.5. Sucrose/D-Glucose/D-Fructose analysis

'Sugars' is the collective term for all sorts of monosaccharides and disaccharides, which are found in nature and are added to food (Institute of Food Science + Technology, 2016). These sugars include glucose, fructose, lactose and sucrose. Sugars are known to play structural roles and serve as substrates in respiratory reactions or intermediate metabolites in many other biochemical processes of plants (Ciereszko, 2018). During photosynthesis, plants use carbon dioxide, sunlight and water to produce oxygen and glucose. The glucose, in turn, serves as food for nourishment in the plant among many other functions. Three types of sugars were investigated in this appraisal, namely, D-Glucose, D-Fructose and Sucrose (Table 22). Glucose is a monosaccharide (simple sugar) which is generated through photosynthesis. It is the most essential monosaccharide for metabolism and one of the primary sources of energy in animals and plants. In plants, the simple sugar is important for plant growth, flower formation, as well as the development of fruits and seeds (Garner, 2019). Of the three sugars investigated in this study, D-Glucose yielded the highest results. D-Glucose averaged 2.99 mg/g among the cultivars (Table 22). Significant ($p < 0.001$) differences were observed among the cultivars, with Nudosa (4.09 mg/g) and Malta (3.58 mg/g) having yielded significantly higher glucose contents as compared to Fresno, Fusicaulis, Meyers and Morado, while Morado (2.33 mg/g) yielded the lowest D-Glucose content out of all of the cultivars of the study. No significant differences were observed in the D-Glucose content of Fresno (2.67 mg/g) and Fusicaulis (2.87 mg/g), nor were they observed between Meyers (2.38 mg/g) and Morado. Nopalitos are young stems, as such, it is highly possible that they would not contain that high of a concentration of glucose as compared to older cladodes whose glucose content can go as high as 190-200 mg/g as reported by Du Toit (2017). This may suggest that glucose levels in plants tend to gradually increase as plants grow. This is further supported by the observation that was made by Ribeiro *et al.* (2010), who reported the content of sugar to being almost double in tertiary (old) cladodes as compared to quaternary (young) cladodes.

Fructose is a simple sugar which is similar to glucose in terms of chemical formula, however, is different from glucose in its molecular structure (Food Insight, 2009). Fructose contributes to the sweetness of plants and is utilised in the production of sucrose. This particular sugar had an average of 2.30 mg/g among the various nopalito cultivars of this study. Significant ($p < 0.001$) differences were observed among the nopalitos (Table 22). Fusicaulis reported a significantly ($p < 0.001$) higher fructose content (3.39 mg/g) as compared to Fresno, Malta, Meyers, Morado and Nudosa, while Meyers had a significantly ($p < 0.001$) lower fructose content (2.03 mg/g) out of all the various cultivars. No significant differences were observed in the fructose content that was found in Nudosa (2.72 mg/g) and Morado (2.75 mg/g). Fresno (2.30 mg/g) reported an equal fructose content to that of the average of the cultivars while

Malta (2.98 mg/g) had a higher fructose content than the average of all cultivars in addition to Nudosa, Morado and Fusicaulis. Naturally, vegetables have minimal amounts of glucose and fructose compared to fruits. For example, the Australia New Zealand Food Authority (1999) reported contents of fructose found in common Australian vegetables such as green beans (0.2 g/100g FW), asparagus (0.8 g/100g FW) and common peeled cucumber (1.1 g/100g FW). These three common vegetables are often closely associated with nopalitos, and from the results obtained in this study, one can deduce that their fructose contents fall within the range of green beans.

Sucrose which is made up of glucose and fructose is the primary transport form of assimilates in plants (Ciereszko, 2018). Due to its ease of extraction from plants, it has been regarded as an important kind of sugar for human beings (Biologydictionary.net Editors, 2018). The cultivars in this study had an average of 0.26 mg/g of sucrose (Table 22). Malta (0.01 mg/g) and Meyers (0.01 mg/g) yielded the lowest sucrose contents as compared to the rest of the nopalito cultivars in the study while Morado (0.67 mg/g) yielded the highest sucrose content. There were no significant differences between the cultivars with regards to the sucrose content. However, an interesting trend was observed in the sucrose values of all cultivars as they were relatively low and almost approaching the zero mark. The values that were obtained during the analysis were a trademark of this specific kit that was used for analysis. Furthermore, the low sucrose values that were observed may be a result of the hydrolysis action that takes place in the plant as sucrose is broken down into glucose and fructose during respiration. The hydrolysis process often results in the sucrose content becoming zero. What was interesting to observe in these results was that there was a clear inverse relationship between sucrose and the two monosaccharides in which it breaks down into, which was evident in all the cultivars. This may be attributed to the rate in which the sucrose molecules broke down during hydrolysis. Ribeiro *et al*. (2010) reported similar sucrose trends when studying the carbohydrates found in cladodes. The researchers observed sucrose only in hydrolysed extracts, which was an indication that sucrose was only found in trace amounts in contrast to glucose and fructose.

5.3.6. Starch analysis

Starch is a polysaccharide that is made up of individual glucose chains and plays a role in storing energy in plants. Human beings cannot synthesise the polysaccharide, as such, once consumed, it becomes broken into glucose units to supply energy while the unused starch remains are stored as fat deposits (Petersen, 2018). The starch content of the various nopalito cultivars averaged 1.7 g/100g (Table 22). Significant ($p < 0.001$) differences were observed among the cultivars. Fresno (2.93 g/100g) and Malta (1.57 g/100g) yielded significantly higher contents of starch as compared to Morado, Meyers, Fusicaulis and Nudosa, while Morado yielded the lowest starch content (0.01 g/100g). No significant differences were observed between Fusicaulis (0.21 g/100g), Meyers (0.21 g/100g), Nudosa (0.57 g/100g) and Morado. Many authors have reported on the role of sucrose in the synthesis of starch. The interesting relationship between the two carbohydrates seems to be noticeable in the obtained results. For one, Morado which had the highest sucrose content yielded the least amount of starch. On the other side of the spectrum, Malta which yielded one of the lowest sucrose contents in the study, yielded the second-highest starch content in the study. The results of the two may be ascribed to the starch sucrose interconversion that takes place in different developmental stages of plants (Owlgen, 2019). It is in this process, whereby the plant either synthesises sucrose and converts it into starch, or synthesises starch and converts it into sucrose. The synthesis and conversion of these carbohydrates are dependent on the needs of the plant at that specific developmental stage. From this, the converted carbohydrates are then transported and stored into various tissues to fulfil various functions in the plant. Figure 18 depicts the interconversion that was observed in the nopalito cultivars of this study. From this figure, one can observe the existence of an inverse correlation between the two types of carbohydrates, whereby one increases as the other decreases. This observation further supports the existence of the starch sugar interconversion in nopalitos.

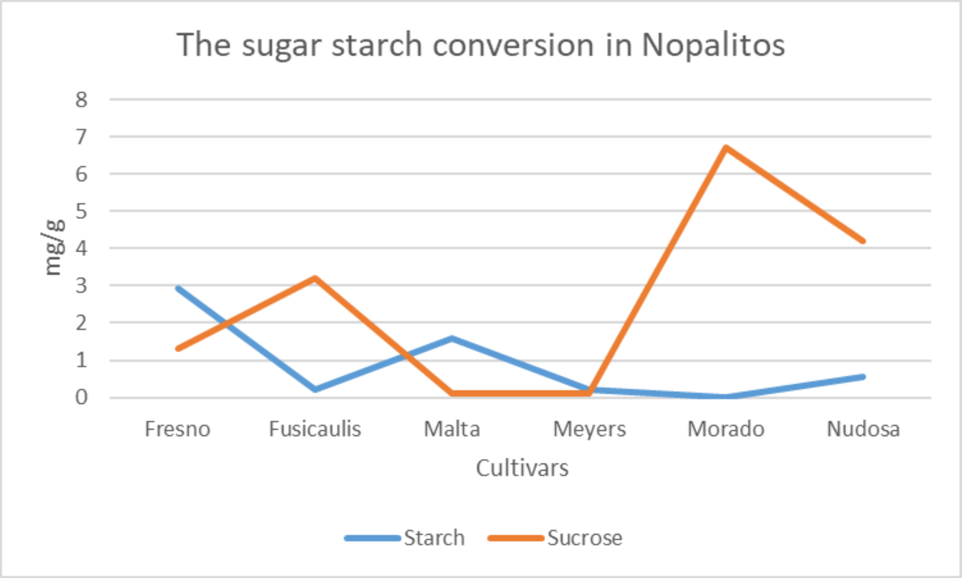

Figure 18: The starch sugar interconversion found in the six different nopalito cultivars

5.3.7. Phytochemicals analysis

5.3.7.1. Polyphenol analysis

The secondary metabolites in plants (polyphenols) serve many functions. For example, they have strong antioxidant and anti-inflammatory effects, both of which can be beneficial for the prevention of cancer (Zhang and Tsao, 2016). Gallic acid is a common polyphenolic acid which was used as a standard in this study. As such, the subsequent polyphenol analysis results from the six nopalito cultivars were expressed as milligrams of gallic acid equivalents per gram (mg GAE/g). The metabolites which were investigated in this study averaged 2.55 mg GAE/g (Table 22). Significant ($p < 0.001$) differences were observed among the cultivars. The results showed Meyers to have a significantly higher polyphenol content (3.17 mg GAE/g) as compared to the remaining five cultivars. This was followed by Malta (3.05 mg GAE/g), while Fusicaulis yielded the lowest content (1.90 mg GAE/g). Nudosa (2.21 mg GAE/g) and Fusicaulis (1.90 mg GAE/g) were lower than the average of the cultivars. Moreover, no significant differences were observed between Fusicaulis and Fresno (1.92 mg GAE/g), nor were they observed between Morado (3.03 mg GAE/g), Malta (3.05 mg GAE/g) and Meyers. The results obtained in this study are similar to some of the individual polyphenol compounds that El-Mostafa *et al.* (2014) found when investigating the different kinds of polyphenols found in cladodes. These polyphenols included gallic acid (0.64–2.37 mg/100g), 3,4-dihydroxybenzoic (0.06–5.02 mg/100g) and 4-hydroxybenzoic (0.5–4.72 mg/100g).

5.3.7.2. Chlorophyll analysis

Good quality nopalitos are regarded as fresh-looking, and turgid with a bright green colour (Guevara *et al.,* 2001). Chlorophyll is the main pigment used by organisms for photosynthesis which is found in chloroplasts and absorbs and reflects specific wavelengths of light (Martin, 2019). Albeit, there are six types of green pigments that one can find, the main ones are chlorophyll-a and chlorophyll-b which were investigated in this study. Martin (2019), has described the pivotal function of chlorophyll-a as being that of a primary electron donor in the electron transport chain part of photosynthesis. The energy that is obtained from the sun is ultimately converted to chemical energy that can be used by the organism for various cellular processes. Chlorophyll-a averaged 2.90 DEE mg/g in this study (Table 22). Significant ($p = 0.002$) differences were observed among the nopalito cultivars. Of the six cultivars, Meyers yielded a significantly ($p = 0.002$) higher chlorophyll-a content (6.40 DEE mg/g) as compared to Malta, Fresno, Fusicaulis, Morado and Nudosa. On the other side of the spectrum, Nudosa yielded the lowest chlorophyll-a content (1.53 DEE mg/g). This means that the chlorophyll-a content that is found in Meyers is a better electron donor in the electron transport chain during photosynthesis as opposed to the chlorophyll-a content that was found in Nudosa. No significant differences were observed between Fusicaulis (2.00 DEE mg/g) and Morado (2.10

DEE mg/g) nor were they observed between Malta (2.68 DEE mg/g) and Fresno (2.69 DEE mg/g).

The primary function of chlorophyll-b is to broaden the absorption spectrum of organisms so that they can absorb more energy from the higher-frequency blue light part of the spectrum (Martin, 2019). As such, the organisms can convert a wider range of energy from the sun into chemical energy. Chloropyll-b averaged 1.64 DEE mg/g in this study (Table 22). Significant (p = 0.003) differences were observed among the cultivars. Nudosa was observed to have yielded a significantly (p = 0.003) higher chlorophyll-b content (5.44 DEE mg/g) as compared to the remaining five cultivars, while Meyers yielded the lowest chlorophyll-b content (0.01 DEE mg/g). This means that the chlorophyll-b content that is found in Nudosa would result in higher chemical energy in the stems as opposed to that found in Meyers. No significant differences were observed between Malta (0.73 DEE mg/g) and Fusicaulis (0.90 DEE mg/g) nor were they observed between Fresno (1.24 DEE mg/g) and Morado (1.53 DEE mg/g). An apparent inverse relationship exists between the two types of chlorophyll found in the various cultivars; an increase of chlorophyll-a leads to the decrease of chlorophyll-b content as seen in Meyers and Nudosa.

Accessory pigments are pigments that facilitate the absorption of various colours on the light spectrum (Dowd, 2019). One such example of a group of accessory pigments is the carotenoids which reflect orange, red and yellow light waves. In the leaves, they form clusters next to chlorophyll-a molecules to give off absorbed photons aptly (Dowd, 2019). In this study, carotenoids averaged 0.84 DEE mg/g (Table 22). Significant ($p < 0.001$) differences were observed among the cultivars. Meyers was observed to have yielded a significantly ($p < 0.001$) higher carotenoid content (1.58 DEE mg/g) as compared to the remaining five cultivars, while Malta yielded the lowest content (0.06 DEE mg/g). This means that the carotenoids found in Meyers would facilitate the absorption of various colours on the light spectrum better than in Malta. Of all the pigments which were investigated in this study, carotenoids yielded the lowest overall pigment content.

5.3.7.3. Flavonoid analysis

Flavonoids are a diverse group of plant chemicals which are responsible for the bright colours in fruits and vegetables along with carotenoids (Szalay, 2015). Furthermore, it has been reported that diets which are rich in these plant flavonoids offer powerful anti-inflammatory and immune system benefits (Szalay, 2015). Cladodes have been reported to contain numerous flavonoids, especially quercetin 3-methyl ether, which is a highly efficient radical scavenger (Lee *et al.,* 2002; Stintzing and Carle, 2005). In this study, the flavonoids that were found in the six nopalito cultivars averaged 0.24 mg QE/g (Table 22). There were no significant differences observed between the different cultivars. Meyers yielded the highest flavonoid content (0.30 mg QE/g) while Fresno and Fusicaulis yielded the lowest flavonoid content (0.20 mg QE/g). The results observed in this study are well within the values which have been reported previously as various flavonoids found in cactus plants differ in amounts and have ranged from as little as 0.22 mg/100g in flavonoid components such as kaempferol (El-Mostafa *et al.,* 2014).

5.3.7.4. Ascorbic acid (Vitamin C) analysis

Recent studies have shown that fruits and vegetables can aid in healing certain diseases, and this can be ascribed to the presence of high phytochemical content and antioxidant capabilities. Ascorbic acid (Vitamin C) is known to be an essential micronutrient that is necessary for the normal metabolic function of the body (Jaffe, 1984), among other functions. In this appraisal, the average Vitamin C content was 130.36 mg/100g (Table 22). Significant ($p < 0.001$) differences were observed among the nopalito cultivars. Fusicaulis yielded a significantly ($p < 0.001$) higher Vitamin C content (141.67 mg/100g) as compared to the rest of the cultivars of this study. This was followed by Fresno (136.82 mg/100g), while Nudosa yielded a significantly ($p < 0.001$) lower content (115.50 mg/100g) compared to all cultivars. No significant differences were observed between Meyers (124.22 mg/100g), Morado (131.01 mg/100g) and Malta (132.95 mg/100g) nor were differences observed between Fresno and Fusicaulis. What was most interesting about the findings was that the Vitamin C content of the six cultivars is easily comparable to that of common vegetables as well as fruits and even higher than some of them. For example, oranges (45-60 mg/100g), strawberries (40-90 mg/100g), broccoli (113 mg/100g) and brussels sprouts (109 mg/100g) all have lower reported Vitamin C contents than the nopalitos in this study (Davies *et al.,* 1991). Furthermore, the values were significantly higher than those of the cladodes (7-22 mg/100g) which were reported by El-Mostafa *et al.* (2014).

5.3.8. Antioxidant activity

Antioxidant activity is defined ‘as and limitation of the oxidation of proteins, lipids, DNA or other molecules that occurs by blocking the propagation stage in oxidative chain reactions’ (Huang *et al*., 2005). Primary antioxidants directly rummage free radicals, while secondary antioxidants inhibit the formation of free radicals indirectly through Fenton’s reaction (Atasoy *et al.,* 2019). Ways in which this activity was measured in this study were through Ferric Reducing Antioxidant Power (FRAP) and Oxygen Radical Absorbance Capacity (ORAC).

5.3.8.1. FRAP (Ferric Reducing Antioxidant Power) analysis

FRAP assay is a widely used method that uses antioxidants as reductants in a redox-linked colourimetric reaction, wherein ferric iron (Fe3+) is reduced to ferrous iron (Fe2+) at low pH which causes the formation of a coloured ferrous-probe complex from a colourless ferric-probe complex (BioVision Incorporated, 2020). In this study, the average FRAP content was 1.73 µmol AAE/g (Table 22). Significant ($p < 0.001$) differences were observed among the cultivars. Meyers recorded a significantly ($p < 0.001$) high FRAP content (2.01 µmol AAE/g) which translates to its ability to rummage free radicals as compared to Fresno, Fusicaulis, Morado, Malta and Nudosa. This was followed by Morado (1.88 µmol AAE/g), while Fresno was observed to have a significantly ($p < 0.001$), lower content (1.46 µmol AAE/g) than the remaining cultivars of the study. The FRAP content that was lower than the average of the six cultivars was observed in Fresno, Nudosa (1.55 µmol AAE/g) and Fusicaulis (1.70 µmol AAE/g) while that which was found in Morado (1.88 µmol AAE/g), Malta (1.79 µmol AAE/g) and Meyers was above the average of all of the cultivars. Boutakiout *et al.* (2018) investigated the antioxidant activity of juice extracted from cladodes of the Opuntia *ficus-indica (*spineless) and Opuntia *megacantha* (spiny) genotypes, using a FRAP assay. From the FRAP assay results that they obtained, the researchers observed the average antioxidant capacity of the cladode juice from the spineless cultivar to be 2.65 µmol TE mL^{-1} while the spiny cultivar averaged an antioxidant activity of 2.87 µmol TE mL^{-1}. They further reported that though found in small amounts, quercetin which is a powerful antioxidant was responsible for much of the antioxidant activity of cladodes. Barreca *et al*. (2011), have observed the antioxidant activity of lemon juice (0.32 µmol TE mL^{-1}) to be lower than that of cladode juice.

5.3.8.2. ORAC (Oxygen Radical Absorbance Capacity)

The ORAC assay depends on free radical damage to a fluorescent probe, of which fluorescein is the most commonly used probe (Ou *et al.,* 2001). The damage comes as a result of an oxidising reagent which leads to a progressive loss of fluorescent intensity. There exists a correlation between the resulting damage and the amount of oxidant present (Determination of Antioxidant Potential using an Oxygen Radical Absorbance Capacity (ORAC) Assay with Synergy™ H4, 2012). Contrariwise, a correlation between the inhibition of oxidative damage to the fluorescent probe and the antioxidant capacity of a compound which acts as a free radical scavenger exists (Determination of Antioxidant potential using an Oxygen Radical Absorbance Capacity (ORAC) Assay with Synergy™ H4, 2012). In this study, the average of the ORAC content was 89.41 µmol TE/g (Table 22). Significant ($p < 0.001$) differences were observed among the nopalito cultivars. Malta recorded a significantly ($p < 0.001$) higher ORAC content (99.97 µmol TE/g) as compared to the five remaining cultivars. This was followed by Meyers (99.09 µmol TE/g), while Fusicaulis had the lowest content (75.51 µmol TE/g). No significant differences were observed between Fresno (83.34 µmol TE/g) and Fusicaulis, nor were there any differences observed between Meyers and Malta. The ORAC content that was found in Fusicaulis, Fresno and Nudosa (84.04 µmol TE/g) was observed to be below the average of all of the cultivars while that which was found in Morado (94.52 µmol TE/g), Meyers and Malta was above the average. When comparing the ORAC values that were obtained in this study to those of common vegetables reported by Ou *et al.* (2002), it was observed that all six nopalito cultivars recorded values higher than peas (12-29 µmol TE/g) and were more or less in the same range as carrots (25-99 µmol TE/g). When comparing the FRAP and ORAC results, one can deduce that the latter was higher; as such, the nopalito cultivars in this study have stronger antiradical activity than antioxidant activity.

5.4. Conclusion

The *Opuntia ficus-indica* species of the cactus pear plant's use and consumption have increased over the years because of its high nutrient capacity. This is ascribed to the high content of vitamins, proteins and minerals that this species of plants presents. The study proved the high quality and nutrient content of the various cultivars, which was easily comparable to common fruits and vegetables such as soybeans, nuts, oranges and seeds. To identify the possible best overall cultivar, a table was created to show all parameters which were investigated in this study and rank the best cultivar for each of those parameters (Table 24). From this table, the most frequently ranked cultivar was regarded as the best cultivar. As such Malta (14) was deemed as the best cultivar, followed by Fusicaulis (10) and Meyers (9).

Table 24: An indication of the highest nutritional contents found among the six cultivars that were investigated in this study

Parameter	**Cultivar**					
	Fresno	**Fusicaulis**	**Malta**	**Meyers**	**Morado**	**Nudosa**
Crude protein (High content required)			✓			
NDF (Low content required)		✓				
ADF (Low content required)			✓			
ADL (Low content required)	✓					
Ca (High content required)	✓					
Mg (High content required)	✓					
Na (High content required)			✓			
K (High content required)		✓				
P (High content required)					✓	
Starch (High content required)	✓					
Glucose (High content required)						✓
Sucrose (High content required)					✓	
Fructose (High content required)		✓				
Vitamin C (High content required)		✓				

Chlorophyll-a (High content required)				✓		
Chlorophyll-b (High content required)						✓
Carotenoids (High content required)				✓		
Polyphenols (High content required)				✓		
FRAP (High content required)				✓		
ORAC (High content required)			✓			
Flavonoids (High content required)				✓		
%Fat (Low content required)						✓
%FFDM (High content required)			✓			
%Moisture (High content required)					✓	
FAME (%total fatty acids):						
Myristic (Low content required)			✓			
Palmitic (Low content required)			✓			
Palmitoleic (High content required)			✓			
Margaric (Low content required)		✓		✓		
Heptadecenoic (High content required)		✓				
Stearic acid (High content required)		✓				
Oleic (High content required)				✓		
Vaccenic (High content required)						✓
Linoledaidic (High content required)					✓	
Linoleic (High content required)			✓			
Arachidic (Low content required)					✓	
α-Linolenic (High content required)			✓			
Eicosatrienoic (High content required)				✓		
Lignoceric (Low content required)		✓				
Fatty acid ratios:						
Total Saturated Fatty Acids (SFA) (Low content required)			✓			
Total Mono Unsaturated Fatty Acids (MUFA) (High content required)				✓		
Total Poly Unsaturated Fatty Acids (PUFA) (High content required)			✓			

Total Omega-6 Fatty Acids (n-6) (Low content required)		✓				
Total Omega-3 Fatty Acids (n-3) (High content required)			✓			
PUFA:SFA (High content required)			✓			
n-6:n-3 (Low content required)		✓				
Total for each cultivar	**4**	**10**	**14**	**9**	**5**	**4**

Chapter 6

6.1. General discussions and conclusions

The current state of food security and the added strain on agricultural resources the world over, warrants for the investigation, exploration and development of new ways and products to meet the demand of feeding the population. The extensive research evidence that has been presented on nopalitos is of paramount importance as they add value to the cactus plant as a sustainable, multifaceted crop. These young stems that have been consumed as a staple vegetable in Latin American countries for several years, are adorned for their nutritional and medicinal properties. For a vegetable that presents such highly desirable quality and properties, it is concerning that little is still known about it in some parts of the world. One such country is South Africa, whose focus has been predominantly on the cultivation and utilisation of the fruit and the seeds of the cactus pear plant, often neglecting the potential that the cladodes possess. It is as such that this study's focus area was these young stems and how gathering extensive information on them would further support them being considered as a commercial commodity by the agricultural sector and overall consumers of the country.

The 20 South African nopalito cultivars that were chosen for this specific study were derived from a recent study by Du Toit (2017), who conducted the most thorough investigation on the properties and characteristics of mucilage of cladodes that were obtained from 42 South African cactus pear cultivars. It is in that study whereby, the author managed to categorise the 42 cultivars according to their level of mucilage yield. This became a predetermining factor in the selection of the 20 nopalito cultivars that featured in this current study. Because consumers prefer nopalitos which have the least amount of mucilage as this affects their eating qualities, the twenty cultivars of this study were selected according to that criteria.

This particular study evaluated the various characteristics of the 20 nopalito cultivars in terms of their morphology, physiological, physico-chemical properties, physical properties, chemical properties, nutritional properties as well as their sensorial properties and overall eating quality and acceptability. The ideal commercial raw nopalito must be thin, and turgid, look fresh in appearance, and have a bright green colour. Moreover, they must be preferably low in acidity and mucilage content as these two characteristics have a negative influence on the eating qualities of the vegetable. These qualities served as a frame of reference when analysing and comparing the various cultivars to quantify which ones were the best.

The influence of cultivar on the morphological and physiological attributes revealed that some characteristics were influenced more than others. Because the nopalitos were harvested in the recommended consumable length (i.e., 12-20 cm), this parameter was easily comparable among the different cultivars over the two years. The heaviest cultivars that were observed under this investigation were Nudosa, Robusta, Fusicaulis, Santa Rossa and Fresno, while the thinnest cultivars were Ofer, Morado, R1251, Malta and Turpin. The thickest cultivars were Fusicaulis, Fresno, Robusta and Nudosa, while the thinnest cultivars that were observed were Santa Rossa, Gymno Carpo, Morado, R1251 as well as Ofer. Interestingly enough, with the exception of Santa Rossa, the thickness of the heavier cultivars was higher than that of the less heavy cultivars. A similar trend was observed in the amount of extractable mucilage from the various cultivars whereby, the heavier cultivars (Fusicaulis, Fresno, Nudosa, Robusta and Santa Rossa) yielded some of the highest amounts of mucilage as opposed to the likes of Ofer, Morado and Malta which yielded low amounts of mucilage. This parameter was important to investigate as it directly affects the eating qualities of the vegetable, because too high of a level of mucilage presents sliminess which is regarded as undesirable by many consumers.

The analysis of the effect of the different cultivars on the physical attributes showed that the softness and flexibility of the different cultivars vary. It is stated that high values of firmness indicate thin and flexible nopalitos while low values of firmness indicate inflexibility or tough nopalitos. From the results obtained it was evident that the statement was true as the thinnest cultivars such as Ofer, Morado and Gymno Carpo yielded the highest values while the thickest cultivars such as Nudosa, Robusta and Fresno yielded the lowest values of firmness which translates to them being the least flexible cultivars in the study. This suggests that there may exist a relationship between the thickness and firmness of the nopalitos. Two methods were employed to investigate the viscosity of the mucilage that was extracted from the 20 cultivars. The results of this study proved Du Toit's (2017) observation in that the line-spread method of determining viscosity proved to provide the most quantifiable data as opposed to other methods such as using the viscometer, which was in this case, the second method of determining viscosity among the cultivars. From an eating quality perspective, nopalitos which had the least viscous mucilage were regarded as the best, as such, the likes of Gymno Carpo, Meyers, Turpin, R1260 and Fresno were regarded as the best cultivars in the context of this attribute in the study. The investigation of the colour properties of the nopalitos revealed that the 20 cultivars are of high quality. The luminosity (L*) values indicated that the brightness of the nopalitos was medium range. The a* values of the cultivars resembled a green colour as opposed to that of red, while the b* values indicated that the cultivars possessed a yellow undertone as opposed to a blue one. Furthermore, the Chroma (C*) values illustrated high intensity of tones, while the mean h˚ indicated the cultivars to be yellow-green.

Because the nopalitos were harvested at an optimal time of the day, the acidity of the various cultivars was more or less in the same range to the extent that the minor differences would not necessarily be distinguished by the majority of consumers. The turgidity of the nopalitos was of a high standard which can be attributed to the high moisture content that was observed in the various cultivars.

With every passing year and planting season, comes a number of modifications that take place in these plants that may directly and indirectly affect the various attributes and ultimately the quality of the harvest. The modifications can be attributed to parameters such as climatic and agricultural changes. It was as such, necessary to investigate how the attributes would compare when harvested in two years, which were 2018 and 2019 in this case. The attributes which were mainly affected by the year of harvest were the colour coordinates of the nopalitos, the mucilage extraction process, the softness and flexibility as well as the acidity of the different cultivars, much of which were more favourable in the 2018 harvest as compared to the 2019 harvest.

The evaluation of the effect of cultivar and the year of harvest interaction on the nopalitos was of the utmost importance as it provides insight into formulating the best planting and harvesting practices, to ensure maximum quality yield in each and every season. The differences of each cultivar were evident in the mucilage extraction process, viscosity, acidity, sugar content as well as the colour coordinates of the cultivars. The results obtained under this investigation correlated with the results obtained under the aforementioned investigations. It was also interesting to observe that the reported work was easily comparable to commercially world grown cultivars such as ‘Atlixco’ and ‘Milpa Alta’.

The cultivars that were of great interest as a result of the variations that were found over the two years, were Meyers, Malta, Nudosa, Fusicaulis, Fresno and Morado. These aforementioned cultivars were further investigated for their nutritional properties in the follow-up chapter. The nutritional analyses of the six cultivars revealed that the incorporation of nopalitos into one’s daily dietary regime could be as beneficial as any other common vegetable. This is because of the high quality and quantity of nutritional elements which were easily comparable with those found in other fruits and vegetables. For example, the Vitamin C content of the six nopalito cultivars was higher than that found in the likes of oranges, brussel sprouts and strawberries. The low-calorie nature of the nopalitos was evident in the low-fat content and high moisture that they presented. All in all, the six nopalitos showed exceptional nutritional properties, with Malta, Fusicaulis and Meyers being the cultivars to have exhibited the most favourable properties, respectively.

The sensory profiling of the nopalitos from 20 cactus pear cultivars, using the Check-All-That-Apply (CATA) technique provided insights into the overall perception and acceptability of the cultivars as a staple vegetable. Out of the 20 cultivars, Skinners Court ranked the highest in terms of overall liking on the 9-point hedonic scale. Compared to the control vegetables i.e., cucumber and green pepper, the nopalitos were the least preferred, with the cultivar Robusta being the least preferred as it presented undesirable eating qualities such as sliminess and thickness.

From the various analyses that were conducted in this study, it was evident that there is no single cultivar that encompasses all favourable traits. However, the impressive results that were observed, deem it necessary for the commercialisation of nopalitos in the South African markets with the leading cultivars being Malta, Fusicaulis, Meyers and Morado.

6.2. Recommendations for further study

Future research of the young cactus stems must include product development, which will look into various methods of preparation of the stems and incorporation into different recipes as well as products in order to increase the overall acceptability, palatability and the masking of undesirable traits of nopalitos for the South frican consumer. Furthermore, a thorough cost analysis must be conducted in order to ascertain the financial implications that would come with the cost of production of the nopalitos on a commercial scale in the country.

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