ANTIOXIDANT CONTENT AND POTENTIAL OF FRESH AND PROCESSED CLADODES AND FRUIT FROM DIFFERENT COLOURED CACTUS PEAR (*OPUNTIA FICUS-INDICA* AND *OPUNTIA ROBUSTA*) CULTIVARS

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Declaration

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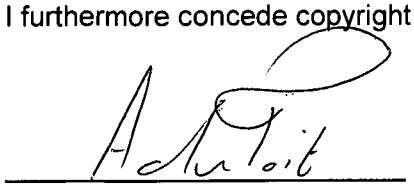

This dissertation is dedicated to my mother, Marie Viljoen, who bravely fought and beat cancer during the time that I was researching this work and to my father David Viljoen to whom I am eternally thankful for standing by her during her illness and treatments.

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Glossary of abbreviations

Abbreviation	Description
%	Percentage
°C	Degrees Celcius
µg	Microgram
µmol/g TE	Micromole of Trolox equivalents per gram
ABST	3-ethelbenzothiazoline-6-sulfonic acid
ANOVA	Analysis of variance
ATP	Adenosine-5'-triphosphate
AVG	Average
CAM	Crussulacean Acid Metabolism
CV	Coefficient variance
DPPH	2,2'Diphenyl-1-picryl hydrazyl
dw	Dry weight
E	East
fw	Fresh weight
g	Grams
g/100g	Gram per hundred gram
GAE	Gallic acid equivalents
Kcal/g	Kilocalories per gram
kg	Kilogram
l	Liter
LDL	Low-density lipoprotein
m/sec	Minutes per second
mg	Milligram
mg/100g	Milligram per 100 gram
mg/kg	Milligram per kilogram
mg/kg GAE	Milligrams of gallic acid equivalents per kilogram
mg/l GAE	Milligrams of gallic acid equivalents per liter
ml	Milliliter
mM	Micromole
mm	Millimeter
mmol/kg	Micromole per kilogram
mmol/l	Micromole per liter
nm	Nanometer
NS	Not significant
O. ficus-indica	Opuntia ficus-indica
O. robusta	Opuntia robusta
ORAC	Oxygen radical absorbance capacity
PCA	Principal component analysis
RDI	Recommended Daily Intake
ROS	Reactive non radicals

S	South
spp.	species
STD	Standard deviation
TE	Trolox equivalents
TEAC	Trolox equivalent antioxidant capacity
Trolox	Synthetic antioxidant
UV	Ultra violet
var	variety
w/w	Wet weight

Chapter 1
Introduction

The *Opuntia* genus belongs to the Cactaceae family and grows mainly in arid and semi-arid regions due to its efficient use of water. It grows in dry conditions where few other crops would grow. It originates from South America (Mexico), but it grows and is easily cultivated all over the world except in Antarctica. Even though it thrives in marginal soils with poor texture and low pH levels, under high temperatures and with little water, it has the highest biomass production rate of all the overground plants (Stintzing & Carle, 2005). Declining water sources and global desertification in many parts of the world caused researchers to pay special attention to indigenous plants from arid lands in order to find effective food production systems and to explore possible uses in the food, medical and cosmetic industries (Yahia *et al.*, 2009).

Research has revealed that *Opuntia ficus-indica* fruit contains high levels of constituents that give it value on a nutritional and functional basis, such as betalains, taurine, calcium, magnesium and antioxidants (Piga, 2004). Crops with health-promoting and nutritional benefits are gaining momentum for both professionals and consumers and cactus pears fit this trend (Moβhammer *et al.*, 2006a).

An antioxidant is a molecule that is able to reduce, delay or inhibit oxidation of other molecules even when present in very low levels. It therefore protects the body against diseases (Gülçin, 2012). There is overwhelming evidence that components of fruit and vegetables may be protective against oxidative damage. There is a non-nutrient compound of fruit and vegetables, in addition to the vitamins, minerals and polyphenols that seems to have beneficial functions in the human body. Much research has focused on the occurrence of antioxidant molecules to find a link between diets rich in fruit and vegetables and the onset and prevention of oxidative stress related diseases. Recently there has been increased interest in the health-promoting capacity of antioxidants and cactus pears have been investigated in this regard. Recent results by Budinsky *et al.* (2001) showed that ingestion of prickly pear cladodes is effective in lowering oxidation injury and this suggests that the prickly pear plant possesses antioxidant components in the edible stems of the plant as well as the fruit. Tesoriere *et al.* (2004) proved that a diet that includes cactus pear fruit may reduce the risk of age-related and degenerative diseases.

Fruit and vegetables have high levels of antioxidants and therefore it is associated with health and reduced risk of chronic diseases. There is a growing interest among the public for safer, healthier food products and a growing trend of consumers who prefer natural foods and additives such as antioxidants, pigments and preservatives (Gülçin, 2012).

The aim of this study was to investigate the presence and potential of antioxidants in *Opuntia ficus-indica and Opuntia robusta* cultivars found in South-Africa, not only in the raw state but also in processed products.

The fresh and processed fruit and cladodes of different coloured cultivars were analyzed for total phenolics, betalains, ascorbic acid and carotenoids. The study was concluded by analysis of the antioxidant capacity of the various antioxidants by measuring the free radical scavenging activity and by the chelating activity of ferrous ions.

The relationship between the ascorbic acid-, total phenolic-, betalain- as well as carotenoid content and their respective antioxidant capacities were correlated for the different parts of the fresh cactus pear plant, that is, the fruit pulp, cladodes and the by-products, namely the seeds and peel that are normally discarded as waste.

Furthermore, marketable processed products from the different parts of the plant were investigated in the same way, in order to compare the presence and action of above mentioned antioxidants to the fresh products.

Chapter 2

Literature review

2.1 Introduction

Cactaceae are most intriguing plants due to their peculiar adaptations to thrive in times of drought and severe heat when few other plants would survive. These adaptations include CAM (Crassulacean Acid Metabolism), the reduction of leaf tissues and cuticular waxes that cover the cladodes and fruit. The Opuntia's ability to regenerate from the roots, cladodes, fruit, seeds, tissue as well as from grafting is another extraordinary feature. Cactus pear plants have widespread and shallow root systems that absorb water from any source, such as mist or light rain. It has the ability to retain water under unfavourable climatic conditions due to the mucilage content in both the cladodes and the fruit (Feugang *et al.,* 2006).

The *Opuntia ficus-indica* cactus is a xerophyte of about 200 to 300 species that originates from South America (Mexico), but it grows and is easily cultivated all over the world. Worldwide it is cultivated for the delicious fruit and in Mexico the cladodes are widely used as a vegetable. It is an indigenous plant to Mexico and is commercially produced in Mexico, Southern California and Chile. Most studies are done in that part of the world to improve the usefulness of this ecologically adaptive plant. As it grows with low inputs, it could produce cheaper alternatives to the expensive commercial products that are available at present, such as fruit juice, concentrates, powders and other functional ingredients. (Moβhammer *et al.,* 2006a)

The classification of the cactus pears studied in this work is briefly summarized as follows:

Order: Caryophyllales

Suborder: Potulacineae

Family: Cactaceae

Subfamily: Opuntioideae

Genus: Opuntia

Subgenus: Opuntia

Species: *ficus-indica* and *robusta* (L.) Mill. Gard. Dict. Abr. Ed. 8 No 2. 1768 (Scheinvar, 1995)

Research is underway at the Texas Agricultural Experiment Station, where molecular biologists are attempting to produce cactus pears that have the advantage of having no glochids or spines, and also a seed free cactus pear that could easily be made into seed-free puree and pickles, called the "Texas A & M 1308". (Phillips, K D, 1996: agnewsarchive.tamu.edu/stories/SOIL/cactus.htm)

2.2 Background and distribution of *Opuntia ficus-indica*

Evidence suggests that Opuntia was extensively harvested for 9000 years before the arrival of the Europeans to Central America. The Aztecs founded their capital on a site indicated by an eagle sitting on an Opuntia. It was prized from early times as the host for the cochineal scale insect that provided a bright red dye. The plant was taken to Europe by Columbus where plantations were established in Spain (Cadiz) in 1820. It was introduced to the Canary Islands in 1824 where its cultivation became widespread to supply the rest of Europe with red dye. From Spain, the *Opuntia* spread east through Europe especially around the Mediterranean. When the Moors were expelled from Spain in 1610, the plant spread into Northern Africa and from there, to India. It is from India that the Dutch brought it to their new settlement at the Cape of Good Hope (Van Sittert, 2002; Díaz Medina *et al.*, 2007). The plant was used for several purposes; for the production of carminic acid (red dye), for food use and as medicine. Today, it is widely distributed in the semi-arid regions of Mexico, America, Africa, Australia and the Mediterranean basin (Piga, 2004).

2.3 The cactus pear plant in South Africa

In the Republic of South Africa and neighboring countries, cactus pears found highly favourable environmental conditions. Due to the plant's ability to propagate, grow and spread on any soil or grow on rocks, the spiny cactus pear was declared as an invader plant in South Africa. It invaded an estimated 900 000 ha of natural pastures mainly in the Eastern Cape and Karoo. Insect enemies like the cochineal insect and cactoblastis moth were used for the biological control of the plant starting in 1932. Infestations have now been eliminated due to a law, applicable only to the spiny plants, prohibiting the uncontrolled growing. The spiny plant is officially declared a weed in South Africa, thus the commercial cultivation of *O. ficus-indica* in South Africa is limited to the spineless Burbank varieties (Van Sittert, 2002). These Burbank varieties (imported from California in 1914 by the Agricultural Research station of Grootfontein at Middelburg), grow on most farms where they serve as a source of fodder in times of drought.

However, there is a growing realization that it can be a useful plant other than being used for fodder and fruit production (Brutsh & Zimmermann, 1993). Research should be encouraged and backed to find other uses that could expand the utilization of the plant.

In South Africa the cactus pear is cultivated for its production of fruit for the local and European markets. The ripe fruit are harvested from December to March depending on the cultivar and climate. The fruit have a short shelf life of 8-10 days at room temperature, but it can be stored for six weeks at the correct temperature (10 °C) and humidity (90 %) (Joubert, 1993). Traditionally, the use of the prickly pear fruit used to be part of the local food source in the arid areas of the Karoo. This is evident in the use of both the fruit and stems in old recipe books written by Mrs. Winnie Louw and Mrs. Anne Schnell that include notes on the making of soap, using the stems in flower arrangements and preservation of the fruit in the form of jams, pickles and crystallized sweets.

In Haenertsburg in the Limpopo Province in the northern region of South Africa, Terence Untepertinger has more than 60 hectares of cactus pear orchards on his farms and is presently expanding to include more land. From these orchards, fresh cactus pear fruit of *O. ficus-indica* variety "Algerian" are exported under the commercial name of "Consolata". The Consolata Estates export cactus pears mainly to Europe ten months of the year. During peak season that stretches from December to March approximately 25 tons are harvested per day and of that, 19 tons are packed for the export market every day (Limpopo Agribulletin, 2011).

Another large cactus pear exporting business, "Afrigold" belongs to Mr. Doug Reed who farms on land that has been in the family since 1892 in the Mooketsi valley. Cactus pears were chosen to be farmed as it was best suited to the dry climate and little artificial intervention would be needed to ensure a profitable business. The three varieties that are planted on 65 ha on dry land as well as under drip irrigation are Algerian (pink-red coloured fruit), Gymno Carpo (orange coloured fruit) and Morado (white-green coloured fruit). It has been marketed under the name "Afrigold" and "Sundance" for the past twenty years. Cactus pears are exported to Europe, Canada and the East as an exotic fruit (http://dsreed.co.za).

It was concluded in a study done at an experimental orchard outside Bloemfontein, South Africa, that as declining food sources and global desertification increase, the importance of *Opuntia* spp. as an effective food production system both as fruit and as a vegetable (cladodes) should be explored. The Meyers, Roedtan, Gymno Carpo and Robusta x Castillo varieties of *O. ficus-indica* were recommended for fruit production and Meyers was proven to be the most

appropriate cultivar for economical purposes in the study. It was also found that there were large variations between cultivars both as a result of genetics and the environment (De Wit *et al.* (2010).

2.4 Utilization of the cactus pear plant

Fruit and cladodes from *O. ficus-indica* are considered to be of Mexico's most valuable genetic resources because of its effective uses in different types of home industry. It is an important part of the Mexican culture and traditions. The fruit plays a big role in the diet of the people of Mexico and Chile, and traditional food preservation methods are still used in homes today (Sáenz, 2002; Corrales- Garcia, 2009) The fruit is eaten fresh, but the whole plant can be used as food and animal feed in arid regions during droughts, therefore it is known in Southern America as "the bridge of life". The fruit is traditionally preserved in many different ways in Mexico. A unique preservation technique is "Tuna cheese" (Queso de tuna), a dried product made from the concentrated juices, raisins, nuts and pine nuts, as well as other treats made from dried and concentrated pulp mixtures. A traditional home made drink from Mexico, "Colonche" is low in alcohol and obtained by fermenting the cactus pear pulp in wooden barrels. Jams, syrups, canned and frozen cactus pear products were generally made at a cottage industry-scale and by farmers for own use (Moβhammer *et al.,* 2006a).

Prickly pear fruit are usually eaten freshly picked, as it has a limited shelf life. The fairly high sugar content and low acidity give the fruit delicious, sweet but sometimes bland taste. It has a very limited shelf life because of the low acid content and should continuously be in cold storage throughout the marketing process. As the pH values are reported to be between 5.3 and 7.1, it is classified as a nonacid fruit and therefore it is susceptible to microbial invasion (Piga, 2004). Various efforts to reduce post harvest decay have to be carried out, to reduce microbial contamination while maintaining the nutritional and sensory properties. Its functionality therefore lies in processed items and the use of extracts that may be used as additives in the pharmaceutical and cosmetic sectors. The seeds, peel and pulp are currently being investigated in order to find suitable applications (Feugang *et al.*, 2006).

Two of the most common domestic uses of the fruit are as juices and pulps. Due to high contents of amino acids such as proline and taurine and the presence of minerals such as calcium and magnesium, cactus pear juices could be valuable ingredients for sports and energy drinks (Reyner and Horne, 2002; Seidl *et al.*, 2000 in Moβhammer *et al.,* 2006a). Data also

indicated that cactus pear pulp had multiple functional properties and could be used as a good source of nutraceuticals such as vitamin C, betalains, phenolics and taurine. The presence of taurine makes it an exceptional fruit as taurine, a conditional essential nonproteinogenic amino acid, is virtually absent in plants, especially fruit. (El-Samahy *et al.,* 2006)

The possibility of using pigments found in cactus pear fruit is being investigated. Betalains, which include the betacyanins (red-violet colour) and the betaxanthins (yellow colour), are found in cactus pears and are indicated as colourants of low-acidic foods as they are stable in a pH range of 4 to 7. Especially the red pigments are being investigated in order to substitute synthetic dyes in the food and pharmaceutical industry. Nowadays, betalains for food use are extracted from beetroot, which contains up to 50 mg/100 g of betanin. Beetroot is the only allowed source of the red, betalain approved additives for use in food in the United States and in the European Union. Unfortunately earth-like flavour characteristics caused by geosmin and high nitrate concentrations associated with the formation of carcinogenic nitrosamines affects the commercial use of beetroot as a source of red colourants. Beetroot has for many years been considered to be the only edible betalainic source, but in China the *Amaranthus* are already in use. Other cactaceae have stimulated investigations into using cactus fruits as a better souce of betalains. Domiguez-Lopez (1995) found double the amount of betacyanins per 100 g in purple colored cactus fruit than in beetroot. In addition, cactus fruits do not contain geosmin and pyrazines that are responsible for the unpleasant flavours, it shows no toxicity and the pigments do not provoke allergic reactions. Another advantage is that it can be used without certification (Moreno-Alvarez *et al.* 2008). It is thus concluded that cactus pears may be a better source of betalains than beetroot.

Piga (2004) investigated the colouring range of betalains from cactus pears at near neutral pH and found that betacyanins and betaxanthins allow a very wide chromatic interval. Moβhammer *et al.* (2005) stated that by mixing yellow-orange and purple juice as well as isolated betaxanthin and betacyanin fractions, they could produce tailor made hues covering the entire spectrum from bright yellow to blue-purple. Cactus pear concentrates are therefore suitable for colouring yoghurts, ice creams and other fruit preparations such as cereal bars, chocolates, instant products and even meat substitutes. Cactus juices could be used to tailor- make hues in bright yellow to red-purple to red for colouring other fruit juices (Moreno-Alvarez *et al.,* 2008). The purification of the betalains are not required to produce different hues, in fact the pigment was more stable when juice was used. Moβhammer *et al.* (2006b) found acceptable overall pigment retentions of 71-83 % after the reconstitution of semi-concentrated and concentrated juice. The

use of cactus fruit powders may be an excellent way of colouring desserts, fruit or cereal bars, instant dishes and chocolates. It opens new fields of application for cactus pears not only as red colourants but also the yellow-orange betaxanthins are being considered a new source of water-soluble colourants (Moβhammer *et al.*, 2006b).

Frozen puree concentrate could be used to flavour products like ice cream. Gels, jams, canned, dried and frozen slices of fruit are products made from the fruit (Sáenz, 2000). As fruits have a high glucose and fructose content, it may also be considered for the manufacture of high fructose glucose syrup (Moβhammer *et al.,* 2006a).

From the earliest times Mexicans collected the seeds from the cactus pear fruits, dried and ground it into flour and used in combination with lucern and hay for animal fodder (Nobel, 2002). According to Sáenz (2002), oil can be extracted from the seeds. Cactus pear seeds have a high grade of unsaturated acids, with the highest content of linoleic acid (Shongwe, 2011). Thus it may be compared to corn and grape seeds oil (Labuschagne & Hugo, 2010).

In Mexico, the whole cactus stems or pads used for food are known as *nopal* or *pencas*. When the young cladodes are cut into bite sized pieces it is called nopales or nopalitos. As the stems or pads are safe for human consumption, they have always been considered an important nutritional food source in Latin America. It has been nicknamed "the bread of the poor" as it is a readily available source of food and is often eaten as a green vegetable. In fact, the serving of the fresh young and tender cactus pads called "nopalitos" in a dish similar to green beans is deeply embedded in the culture and local cuisine (Feugang *et al.*, 2006). It is prepared either raw in dishes such as salads and salsas or cooked by means of boiling or frying. It is used with other ingredients in a variety of traditional culinary dishes including desserts, beverages, snacks, soups, stews, sauces and salads. Nopalitos are also preserved in brine or pickled (Rodrigues-Felix & Cantwell, 1988). Recipes and notes on how to use nopalitos are widely available in South and North America. Joyce L. Tate's Cactus Cookbook is an example of this (Savio, 1989). Besides as a food source, cladodes have been proven to have many different health benefits. The use of cladodes in phytochemicals have been investigated because of the high content of total phenolics and specifically flavonoids. Therefore using cactus flour in processed products such as tortillas and other type breads as a nutraceutical supplement has received research attention (Santos-Zea *et al.,* 2011).

Sáenz (1996) claims that the most common preserved product made from cladodes is marmalade. In 1986, when Tirado (cited in Sáenz, 2000) investigated jam from cladodes, he

found that the product is not different to other jams on the Mexican market in relation to aroma, colour, taste, texture and appearance. Badillo (1987, cited in Sáenz, 2000) experimented on making jams from cladodes and added either citric acid, lemon peel or lemon juice to lower the pH in order to improve the gelling of the product. It produced a product with good sensory and microbial stability. Cladodes could also be pickled with vinegar, spices, herbs and olive oil with good results.

Villarreal (cited in Sáenz, 2000) made and tested candy made from the cladodes with or without chocolate coatings with very good results. Crystallized cladodes that resemble crystallized melon peel are another product that was well liked by consumers (Sáenz, 2000).

Other goods made from dried cladodes include woven mats, baskets, fabrics and paper. The whole plant is used by growing it into fences by planting them close together to keep out any intruders. Plantings have also been made to control erosion in deforested areas (Savio, 1989). The spineless stems of the *Opuntia* have played a significant role in providing valuable nutrients for farming animals by using it as fodder (Feugang *et al.*, 2006). Another very interesting use of the cladodes was found in Chile where farmers traditionally used the liquid in which nopalitos were cooked (that contained the slimy mucilage), to clarify drinking water. Studies indicated that mucilage from the *O. ficus-indica* cladodes had not only a clarifying, but also a purifying ability similar to the purifying action of aluminum sulfate (Buttice *et al.,* 2010) and could remove arsenic form drinking water (Fox *et al.,* 2010) In Mexico, there is another traditional use of mucilage where it is used in combination with lime to improve the adhesion properties of paint (Cárdenas *et al.,* 1998).

2.5 Medicinal uses associated with cactus pear fruits and cladodes

There is epidemiological evidence that people who eat the Mediterranean-style diet, which is rich in fruit and vegetables, have few incidences of age-related illnesses such as cardiovascular diseases, cancer and neurodegenerative disorders (Livrea & Tesouriere, 2006). The antioxidant components in the fruit and vegetables prevent oxidative stress and therefore could be responsible for long-term health outcomes. Therapeutic properties of the *Opuntia ficus-indica* have for long been known in traditional medicine (Livrea & Tesouriere, 2006). Cladodes, especially, have been used in folk medicine for treatments of gastritis, fatigue, dyspnoe and liver injury; it has been used in rheumatic disorders, erythemas and the treatment of chronic skin infections in Mexico. In European countries such as Spain and Italy, it was used in folk

medicine, for example for the treatment of diabetes as an antidiabetic drug (Diaz Medina *et al.*, 2007).

Stintzing *et al.* (2005) compiled a current pharmacological profile for the *Opuntia spp.* that described the antioxidant capacity, analgestic action, anti-inflammatory properties, antiulcerogenic effect, hypoglycemic and antidiabetic effects. The anti-hyperlipidemic, cholesterol lowering, anti-arhergenic and diuretical effects were also discussed. Further pharmacological effects that were elaborated on were the impact on uric acid metabolism, the anti-spermatogenic, the antiviral properties as well as the monoamino-oxidase inhibition.

Feugang *et al.* (2006) elaborated on the medicinal use of the fruit and cladodes as well and discussed the anti-cancer effect, anti-oxidant properties, anti-viral effect, anti-inflammatory effect, anti-diabetic (type II) effect, anti-hyperlipedemic and hypercholesterolemic effects and agreed that it could be used as treatment to ulcers and rheumatism, as well as function as an antiuric and be used for diuretic treatments.

Livrea and Tesoriere (2006) looked at the health benefits of bioactive compounds in cactus pear fruit and described the decrease of body oxidative stress in humans, the cardiovascular protective effects, the antiulcer and the hemoprotective effect. Extracts from cactus pear fruit were preventative against cancerous tumor growth and in alleviating the excitotoxic neuronal damage induced by global ischemia. The inhibiting effect on lipidoxidation in human red blood cells and the treatment of ovarian, cervical and bladder cancer cells were also elaborated on.

Budinsky *et al.* (2001) proved that prickly pears, besides the already known hypoglycemic, hypolipemic and antiplatelet effects, exerted significant anti-oxidative action in the body. They concluded that cactus pears may be a nutritional option to be used more widely as a cheap therapeutic medication even in patients with severe hypercholesterolemia.

Sreekanth *et al.* (2007) added value to the nutriceutical characteristics of the fruit of *Opuntia ficus-indica*, by proving the anti-cancer effects of the betanin found in fruit. They demonstrated that betanin, isolated from the fruit of *O. ficus-indica*, enters K562 cells and alters the mitochondrial membrane integrity, leading to a cytochrome *c* leakage, the activation of caspases and nuclear disintegration.

Alimi *et al.* (2012) found that the juice of purple cactus fruit was rich in phenolics, flavonoids, ascorbic acid, carotenoids and betalains and displayed intrinsic scavenging activity. It was explained why cactus juice has *in vivo* antioxidant activity against harmful species related to

ethanol abuse and therefore could protect erythrocytes from ethanol injury. Supplementation with cactus juice protected against lipid peroxidation and normalized the impairment of osmotic abilities and morphologic aspects. Therefore purple cactus juice may be used in the treatment of hangovers.

Mucilage is a very important source of soluble fibre and products using the whole cladode contain large amounts of both insoluble and soluble fibre. Fibre is divided into soluble fibre (it will dissolve and swell in water and is then fermented by bacteria in the large intestine) and insoluble (does not dissolve and is not metabolized by bacteria) (Peña Valdivia *et al.*, 2006). The advantage to the digestive system is that the insoluble fibre binds to toxins and the soluble fibre increases stool bulk. Plant polysaccharides such as pectin and mucilage found in cladodes are not hydrolyzed nor absorbed by the human digestive system, but they can make up the greater part of the alimentary fibre (Sáenz *et al.,* 2002).

Since mucilage is soluble dietary fibre, it is associated with decreasing cholesterol levels and control of glucose in the blood. It diminishes the risk of cancer in more than one way. Firstly, it reduces the risk of cancer such as colon cancer because of the capacity to hold water that insures stool bulk. The fermentation of soluble fibre during digestion also produces rapid intestinal transit. Secondly, due to the presence of lignin, dietary fibre also has anti-oxidation properties through the prevention of free radical formation. Fibres from dried cactus and from cactus fibre isolate were tested by Rosado and Diaz (1995, cited in Sáenz *et al.*, 2004) and found to be effective in all above properties. Fibres are used as a main source of prebiotics and lactic bacteria have been used widely as probiotics in foods. Mucilage could therefore act as a prebiotic to promote the growth of probiotic bacteria (Yahia *et a*l., 2009).

Prickly pear cladodes act in a similar way to other products rich in soluble dietary fibre to decrease cholesterol levels. The soluble fibre in the prickly pear decreases plasma LDL levels by increasing apolipprotein B/E receptor expression. (Sáenz *et al.*, 2004) In a study done by Hernández *et al.* (1998, cited in Sáenz *et al.*, 2004), nopal fed to rats proved that it could have a beneficial effect on hypercholesterolemic patients.

In research done by Frati-Munari *et al.* from 1983 to 1990 (cited in Sáenz *et al.,* 2004) into the hypoglycemic properties of *Opuntia ficus-indica*, they found that it acts as an interfering agent in the absorption of intestinal glucose. It functions by reducing absorption through the soluble fibre content of the cactus pad and also by an unexplained hypoglycemic action of the cactus pads. The cladodes clearly have the ability to decrease glucose levels in the blood and can control

experimentally induced diabetes (Gutierrez, 1998). Raminez and Aguilar (1995, in Sáenz *et al.*, 2004) presented in their finding after studying eight different research reports that *Opuntia* has a strong glucose reduction effect. As the control of glucose levels cannot be explained by the presence and action of dietary fibre only, it is said to be the action of the cactus extract that improves utilization of glucose at the cellular level.

Results from a study done by Galati *et al.* (2003) showed that consumption of nopalitos prevented the development of ethanol-induced mucosa by stimulating a protective response from the gastric mucosa in the stomach. According to Sáenz *et al.* (2004) it is possible that mucilage prevents the penetration of the necrotizing agent into gastric mucosa, therefore acting synergically with the natural defense factors of the gastric mucosa and preventing stomach ulcers from forming.

More research is necessary, but Galati *et al.* (2003) indicated that lyophilized cladodes have significant anti-inflammatory properties and it is also suspected that *Opuntia streptachanta* will inhibit replication of DNA and RNA viruses, but the inhibitory component is presently unknown (Ahamd *et al.*,1996 cited in Sáenz *et al.*, 2004).

Mucilage is also used in the treatment of wounds. As it forms a gel, it exerts a cooling effect, which will ease the pain and accelerate healing (Stintzing *et al.*, 2005).

The cactus cladodes are dried and ground and used as a powder in medicine to regulate weight, increase fibre intake or manage diabetes mellitus. The effectivity has not been proven but has exciting prospects as it is a low kilojoule food ingredient with high fibre content. It could be considered a natural food supplement that may be used in solid or liquid food products (Sáenz *et al.*, 2002, Stintzing *et al.*, 2005). High levels of potassium do not occur in many foods, but dried cactus flour is a good natural source for this mineral (2.1 g/100g) and together with the low sodium content it may potentially have significant impact on the nutrition of products that contain a certain percentage of nopal flour (Sáenz, 1997).

Hfaiedh *et al.* (2008) studied the protective effect of cactus cladode extract upon nickel-induced toxicity in rats and found that regular ingestion of cladode juice was able to counteract the peroxidative effect of nickel, suggesting that flavonoids and more particular quercerin in cladode juice provided highly effective radical scavenger effects.

Brahmi *et al.* (2011) investigated young cladodes (2-3 weeks of age) and concluded that it should be considered as an accessible source of natural antioxidants that is hepatoprotective,

as it enhances liver function and showed a total reduction of aflatoxins induced genotoxicity markers. The cladode juice also prevented or scavenged the formation of reactive oxygen species.

Park *et al.* (2010) suggested that *O. saboten* contains two flavonoids (kaempferol and quercitin) that increase β-endorphin that functioned as an important physiological regulator in response to depression. It showed anti-depressant effects in chronically stressed mice, when mice were restrained for 2 hours daily for 14 days. They suggested that it can be developed into a useful remedy for depression treatment. Kim *et al.* (2010) also tested the cladodes of the same *O. ficus-indica* variety and found that subchronic treatment with cladode juice improved long-term memory as it mediated hippocampal signaling pathways and increased survival rate of immature neurons.

2.6 Morphological view

The plant may be divided into the root, vegetative part, fruit and flower (Figure 2.1).

Figure 2.1: Du Toit, A. (Photographer). 2011. The Opuntia ficus-indica plant with flowers. (Photograph).

2.6.1 Fruit (pulp)

The fruit is a fleshy berry; it has various shapes and sizes (Moβhammer *et al.*, 2006a). Fruit are known as prickly pear, tuna or fico d'india and comes in a rainbow of colours from white, green, yellow, orange, red, purple and even brown. The pulp colour may not correspond with the peel and may be canary yellow, orange or red-rose. It is oval shaped and has a thick pericarp (peel) and juicy pulp with many small and hard seeds. The pulp (fruit) contains mainly water (84-90%) and reducing sugars (10-15%), of glucose and fructose in almost equal amounts (Feugang *et al.,* 2006). The large variety of cultivars causes a large variability in data collected from fruit. In general, the thick pericarp accounts for 33% to 55% of the fruit and the soft and juicy pulp for 45% to 67% of the fruit. The weight of the whole fruit ranges from 67 g to 216 g depending on cultivar, origin and climate (Piga, 2004).

2.6.2 Peel

The thick pericarp is covered with small-barbed spines and glochids. The peel is usually between 36 and 48% of the weight of the whole fruit (Moβhammer *et al.*, 2006a). The colour of the peel is not dependant on the colour of the fruit although it usually demonstrates the colour of the fruit (pulp) it may be a different colour altogether.

2.6.3 Seeds

There are considerable variations in form, size, structure, embryo characteristics and testa colour in the cactus pear seeds. Seeds are 10-15% of the edible fruit (Feugang *et al.,* 2006). They are described as hard and bony and may range in number from 120 to 350 per fruit. The seed weight ranges from 2.0 to 7.0 g per fruit (Nobel, 2002). The main ingredients found in the seeds are oils, proteins, fiber and ash. The fiber content is considerably higher than that of other oleaginous seeds such as soybean, peanut and cotton seeds (Piga, 2004).

2.6.4 Cladodes

The stems are composed of a white parenchyma and the chlorophyll- containing parenchyma, which is the photosynthetically active area. It may be covered with spines and hairs or trichomes, forming from the areole, which is characteristic of the cactaceae family. The short, sharp deciduous glochids cover the Opuntia cacti. The areoles are the places where flowers and thus fruit will develop. The cladodes are succulent. Stintzing *et al.* (2005) reported that the cladodes contain carbohydrates (64-71 g/100 g), ash (19-23 g/100 g), fiber (18 g/100 g), protein (4-10 g/100 g) and lipids (1-4 g/100 g).

2.7 Chemical composition of the pulp, peel, seeds and cladodes

The chemical composition of the pulp, peel, seeds and cladodes are shown in Table 2.1 and the revelant physical and chemical characteristics in Table 2.2.

2.7.1 Fruit (pulp)

The fruit has a high pH value of 5.3 to 7.1 and a very low acidity (0.05% to 0.18% in citric acid). The sugars range from 10 °Brix to 17 °Brix and are mainly reducing types, with glucose being the predominant sugar and fructose second, which is the reason for the very sweet taste of the fruit (Piga, 2004). The fruit contains high levels and various numbers of amino acids, such as proline, taurine and serine, eight of which are essential. Vitamin E and β-carotene are present in the lipid fraction of the fruit and seeds. The carotenes and vitamin E improve the stability of the oil through their antioxidant properties. Ascorbic acid is a major vitamin in cactus pears and vitamin B1, B6, niacin, riboflavin and pantothenic acid are present in the fruit (Feugang *et al.,* 2006). The fruit pulp is a good source of minerals especially calcium, potassium and magnesium. The total caloric value is 50 kcal/100 g, which is comparable to that of other fruit such as pears, apricots and oranges. Fruit pulp provide 0.1 – 1.0% oil (Feugang *et al.,* 2006).

Diaz Medina *et al.* (2007) did a chemical characterization of green and orange fruit and found that the consumption of one serving (150 g edible portion) represents an intake of ascorbic acid and total phenolics of 43% and 68%, respectively of the estimations of adequate intakes. In relation to the intake of minerals, the potassium and magnesium contents are moderate with values of nearly 10% for both minerals, but important contributions to the intake of manganese and chromium were observed. The trace elements manganese and chromium have been associated with protection against oxidative damage. One serving of *O. ficus-indica* contributes 20% of the Recommended Daily Intake (RDI) of manganese and 47% of chromium to the human body. The high contribution of chromium as well as the high levels of fibre and other bioactive substances would explain the antihyperglycemic effect of *O. ficus-indica* (Diaz Medina *et al.,* 2007). The relevant physical and chemical characteristics can be seen in Table 2.1.

Table 2.1: The chemical composition of cactus pear pulp, peel, seeds and cladodes.

Component	Fruit	Peel	Seeds	Cladodes
Moisture (g/100 g)	80.45	NA	5.71	91.04
Ash (%)	8.5	12.1	5.9	2.09
Ethanol-soluble Carbohydrates (%)	58.3	27.6	1.59	NA
Starch (%)	4.55	7.12	5.35	1.17
Protein (%)	5.13	8.3	11.8	0.8
Lipid (%)	0.97	2.43	6.77	0.42
Fiber (%)	20.5	40.8	54.2	3.75

NA = data not available

Compiled from Sáenz, 1997; El Kosssori *et al.*, 1998; Bensadón *et al.*, 2010; Shongwe, 2012

2.7.2 Peel

El Kossori *et al.* (1998) reported that the peel contained remarkable amounts of calcium (2.09%) and potassium (3.4%). The findings of Moussa-Ayoub *et al.* (2011) suggested that the bioactive compound isorhamnetin glycoside have been found in *O. ficus-indica* only in the peels of fruit samples. The amount of isorhamnnetin detected in 100 mg of red cactus pear peels was 91 µg/100 mg. These results showed that cactus pear fruit peel is a unique source of isorhamnetin glysocides. The peel provides oil with appreciable amounts of polyunsaturated fatty acids, mainly linoleic acid, α– tocopherol, sterols, β-carotene and Vitamin K_1. Calcium and magnesium are also present in high amounts in the peel (Piga, 2004).

2.7.3 Seeds

The seeds are rich in protein, minerals and sulphur containing amino acids. In a study by Shongwe (2012) it was found that of the 42 cultivars tested from Bloemfontein, the highest oil content was found in *O. ficus-indica* American Giant (8.76%) but that *O. ficus-indica* Meyers demonstrated good oil productivity (7.41%) across three locations and seasons. Therefore Meyers is considered to be the best cultivar for oil production in South Africa. In a further investigation into the stability of cactus oil by Shongwe (2012), three fatty acids, namely stearic acid, oleic acid and behenic acid in the oil was significantly correlated to the oxidative stability index. *O. ficus-indica* Tormentosa was the best cultivar from an oil quality perspective as it had the highest yield together with the best oxidative stability. *O. robusta* spp. Montery and Robusta demonstrated the poorest oil stability.

The seeds are the main source of insoluble fibre and lipids are present in the peel, pulp and seeds. According to the study by El Kossori *et al.* (1998) the seeds contained more protein

(11.8% w/w) than the pulp (5.13% w/w) and peel (8.3% w/w), this indicated low protein content compared to leguminous plants but it was comparable to other food sources such as sweet potatoes. Starch was present only in trace amounts in the seeds, but the lipids content was high at 6.77% w/w. In relation to the fibre content, the seeds were the highest in cellulose (45.1%), and contained less pectin than the pulp and peel. The seeds were also rich in phosphorus and zinc.

2.7.4 Cladodes

The vegetative part is the modified stems (cladodes) that replace the leaves in function (Feugang *et al.,* 2006). Cladodes should be harvested a couple of hours after sunrise when used as food, as they are sweeter, more turgid and higher in vitamins A and C content after a few hours of sunshine. The cladodes are characterized by high malic acid content varying according to CAM rhythm. It contains calcium, magnesium, potassium, phosphorus and trace amounts of iron (Feugang *et al.,* 2006). Younger cladodes show higher carbohydrate, protein and water contents. The juice from cladodes typically has a pH of 4.6 with 0.45% titratable acids and 6.9 g/100 g dry matter. The high calcium and fibre content place cladodes higher than lettuce in nutritional value, but lower than spinach. The calorie content (27 kcal/100 g) is low. According to Nobel *et al.* (1992) the average sugar composition of the mucilage from cladodes is 42% arabinose, 22% xylose, 21% galactose, 8% galacturonic acid and 7% rhamnose. Stintzing *et al.* (2005) concluded that cactus pad hydrocolloids constitute mainly hexoses and pentoses. According to Sáenz *et al.* (2002) the dietary fibre content of cladode or nopal flour is 42.99% of which 28.45% is insoluble and 14.54% is soluble fibre.

Table 2.2: Relevant physical and chemical characteristics of cactus pear fruit.

Parameter		
Whole fruit	Weight (g)	62 to 216
Seeds	Number of seeds/fruit	3 to 7 % of fresh weight
	Hydrocolloids (endospectrum)	arabinans, rhamnogalacturonans
	Total lipids (mg/kg)	98.8 (on dry weight basis)
	Main lipids	linoleic, oleic, palmitic acids
	Main sterols	β-sitosterol, campesterol
Peel	Weight (g)	36 to 48 % of fresh weight
	Colour	green, orange, red, purple
	Hydrocolloids	pectin-like composition
	Total lipids (mg/kg)	36.8 (on dry weight basis)
	Main lipids	linoleic, oleic, plamitic, y-linolenic, ά-linolenic acids
	Main sterols	β-sitosterol, campesterol
	Vitamins (in oil)	Vitamin E
Pulp	Weight (g)	39 to 64 % of fresh weight
	Colour	white, yellow-orange, red, purple
	Main pigments	indicaxanthin, betaxanthin, betacyanin
	Pigment content (mg/kg)	66 to 1140
	pH	5,6 to 6,5
	Main acid	citric acid
	Total titratable acids	0.5 to 1.1
	Total soluble solids (%)	12 to 17 %
	Main sugars	glucose, fructose
	Total sugar content (g/L)	100 to 130
	Sugar: acid ratio	90:1 to 450:1
	Main amino acids	proline, taurine, glutamine, serine
	Main minerals	calcium, magnesium
	Main vitamin	vitamin C
	Hydrocolloids	complex mixture of rhamnogalacturonan and at least 50 % nonpectic substances
	Main lipids	linoleic, palmitic, oleic, y-linolenic, α-linolenic acids
	Main sterols	β-sitosterol, campesterol
	Total lipids (mg/kg)	8.7 (on dry eight basis)
	Main aroma compounds	2-(E/Z)-2,6-nonadien-1-ol, 2-methylbutanoic acid methyl ester

(Feugang *et al.* (2006); Marsuhiro *et al.* (2006); Piga (2004); Ramadan and Mörsel (2003a, b, c); Sáenz-Hernández (1995); Stintzing *et al.* (2005). Compiled by Moβhammer *et al.,* 2006a.)

2.8 The antioxidant content in cactus pear fruit and cladodes

Fruit and vegetables protect against numerous diseases, including cancer, cardio- and cerebro-vascular, ocular and neurological diseases and certain forms of cancer. The protective effect of fruit has generally been attributed to their antioxidant constituents including ascorbic acid, phenolics, betalains as well as carotenoids. It is necessary to identify appropriate foods that contain antioxidants that may protect against free radical damage, LDL oxidation that causes coronary heart disease, platelet aggression and vasodilatation of the arteries as well as DNA damage and cancer. This information will be useful not only for the identification of safe protective food products that are rich in these components, but also for the development of safe food additives (Rice-Evans *et al.,* 1996).

The diversity and variability of cactus pears in different parts of the world is very large and so is the diversity of the fruit contents. The skin and pulp colour, pulp texture, sweetness and flavor of the juice are directly related to the presence, intensity and activity of the nutritional and the functional compounds. The health benefits and nutritional advantages of cactus fruit are closely related to their antioxidant properties that are associated to the presence of ascorbic acid, phenolic and betalain compounds (Yahia & Mondragon-Jacobo, 2011)

2.8.1 Betalains

While most other common fruit, especially red or pink coloured fruit (red grapes, cherry, raspberry, strawberry, peaches and apples) derive their colour from anthocyanins, cactus fruit pigments are betalains (Felker *et al.,* 2008). Moreno-Alvarez *et al.* (2008) defined betalains as a water-soluble nitrogen-containing pigment, which comprise of the red-violet betacyanins and the yellow betaxanthins (Figure 2.2). They are cationized compounds with a positive nitrogen in a polyene system. Betalains are biosynthesized from tyrosine by the condensation of betalamic acid. This reaction results in the formation of the red to violet betacyanins, which is also found in red beets. The condensation of the betalamic acid with an amino acid (e.g. 3-methoxytyramine) results in the formation of the yellow-orange betaxanthins. As in the case of many other plants, betalains are stored in the vacuole as glycosides. The presence of betalains and anthocyanins are mutually exclusive in the angiosperms as both have never been reported in the same plants (Livrea and Tesoriere, 2006; Moreno-Alvarez *et al.* 2008). The colour of cactus pear fruit appear to be more related to betalains than to phenols and carotenoids (Yahia & Mondragon-Jacobo, 2011).

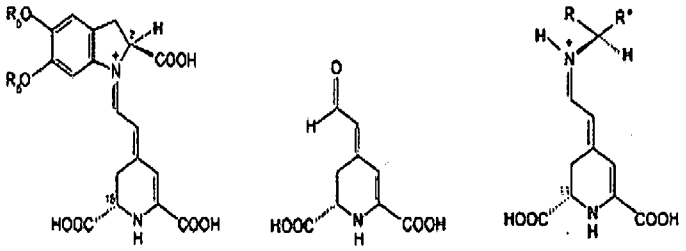


Figure 2.2: Basic structure of betacyanins (left) and betaxanthins (right) and their common building block betalamic acid (middle) (Stintzing & Carle, 2004)

Betalains are of particular interest to food technology because of the potential use as food colourant. In food processing, betalains are less commonly used than anthocyanins and carotenoids although it is stable between pH 3 and 7 that indicates its suitability for colouring low acid foods. Betalains are seen as very stable pigments in processing but is affected by many factors such as: pigment content, the degree of glucosylation or acylation, matrix constituents, chelating agents, antioxidants, temperature, pH, oxygen, light, water activity and nitrogen atmosphere (Moreno-Alvarez *et al.,* 2008). As mentioned before, nowadays betalains for food use are extracted from red beetroot but purple cactus pear fruit such as *O. robusta* have been proven to have double the amount of betacyanins per 100 g and *O. stricta* had five times higher levels than *O. ficus-indica* varieties (800 mg/kg fresh weight) (Moreno-Alvarez *et al.,* 2008). Stintzing *et al.* (2005) found similar contents in the cultivar from South Africa identified as no. 1240 (*O. robusta* from the Burbank variety Chico). It had three times more betaxanthins (yellow pigment) and betacyanins (red pigments) than any other studied cultivar. The sum of both the betacyanins and betaxanthins was more than double that of the nearest other cultivar which was also a purple cultivar from California.

Cactus fruit juice could be used for colouring foodstuff without negative sensorial impact as it tastes pleasant, has no toxicity, does not provoke allergies, are thermally stable, remains stable at different pH levels, showed extended antibacterial stability, showed no non-enzymatic browning and in addition, the plants have minimal soil and water requirements and grow easily in arid and semiarid regions ((Moreno-Alvarez *et al.,* 2008).

Data showed that red and purple fruit contained the highest betacyanin levels, accounting for about 66% of the betalains. The white cultivar showed the lowest content of betalains and contained mostly indicaxanthin (Butera *et al.,* 2002) Similar findings by Stintzing *et al.* (2005); Castellanos-Santiago and Yahia (2008); Figueroa-Cares *et al.* (2010); Sumaya-Martínez *et al.* (2011); Yahia and Mondragon-Jacobo (2011), confirmed that betacyanin content in red and

purple cactus pears was far superior to that of white and yellow fruit. In fact, the purple fruit had up to double that of red fruit.

The study by Butera *et al.* (2002) was the only one in which the yellow cultivar had the highest content of betalains with betaxanthin accounting for 89% of the betalains among yellow, red and white fruit. These findings are not in agreement with other researchers but it can be assumed that since the yellow cultivar is the main and most popular cultivar in Sicily (90% of plantations) the yellow fruit must be of exceptional quality in this part of the world. Butera *et al.* (2002) suggested that the overlapping of betalain absorbance are the cause of inaccurate spectrometric findings by all other researchers who found that purple or red fruit contains the highest betalain levels.

Results from earlier research done by Stintzing *et al.* (2005) suggested that there is a genetic defect in the green varieties that does not permit the formation of either the betaxanthin or the betacyanin pathway. Felker *et al.* (2008) found that the presence or absence of betaxanthins and betacyanins could have a high linkage. They have neither found a purple fruited variety that has betacyanins but no betaxanthins nor a yellow-fruited variety without betacyanins. In contrast, in beetroot there are cultivars with only yellow pigments.

When Moβhammer *et al.* (2006b) tested the betaxanthin and betacyanin contents in cactus pear juice concentrates and powders, it was found that both betalains were stable as there were no changes observed in the contents after heat and storage tests, although microfiltration proved to be a better option than pasteurization. Betacyanins had superior heat stability over betaxanthins. Heat stability would be useful in juice bases and colouring preparations but can also be applied to the stability of betalains as antioxidants. It was found that the addition of 0.1% isoascorbic acid significantly delayed both betaxanthin and betacyanin degradation upon heating. The stability of betalains is influenced by pH, temperature, oxygen, light and water activity (Livrea & Tesoriere, 2006).

Felker *et al.* (2008) stated that the most important commercially distinguishing feature between betalains and anthocyanins is the fading of the anthocyanin pigments towards the neutral pH values when heat was applied, while the betalain pigments continued to absorb strongly at these conditions. Core pigmentation occurs first and before fruit maturity while peel pigmentation only occurs in fully developed fruit upon maturity and epidermal pigmentation seems to occur independently from light stimulation.

It was concluded that regulatory mechanisms independently control pigmentation tissues for inner core, peel and epidermis.

Both betacyanin and betaxanthin compounds behave as scavengers of biologically relevant radicals and antioxidants in various tests *in vitro* and *ex vivo* or may affect redox sensitive cell transduction pathways in cultured cells. Both are also bioavailable (Livrea & Tesoriere, 2006). Betalains are absorbed from the human gut into the systematic circulation in their intact forms, which indicates that hydrolysis is not a prerequisite for their absorption. Betalains are able to go across the red blood cell membranes. The cyclic amine is considered to be the reactive group conferring to this class of molecules reducing properties. Therefore betalains carry a phenolic and an acyclic amine group, which are excellent electron donors and are able to stabilize radicals. On the base of their redox properties, Moreno-Alvarez *et al.* (2008) researched several studies to confirm firstly the antiradical actions of betalians (Butera *et al.,* 2002; Cai *et al.,* 2003; Stintzing *et al.,* 2005), secondly to establish that betalains prevent active oxygen-induced and free-radical-mediated oxidation of biological molecules and thirdly, to confirm that they are able to exert action as antioxidant *in vivo.* It was concluded that the consumption of cactus pear fruit positively affect the body's redox balance and decrease the oxidative damage of lipids as a result of the betalain content in the fruit (Moreno-Alvarez *et al.,* 2008).

It was suggested by Butera *et al.* (2002) that the high antioxidant potential values measured in cactus pears of three different colours suggested the presence of very effective electron donons and/or H-atom donors. It was speculated that the results from their study indicate that betanin and indicaxanthin provided a marked antiradical activity against 2, 2-azinobis (3-ethelbenzothiazoline-6-sulfonic acid) (ABST) cation radicals. In fact, it was found that purified betanin (red) has a tenfold higher Trolox equivalent antioxidant capacity (TEAC) value than the purified indicaxanthin (yellow) extracts. On this basis, the antioxidant capacity of prickly pear was twice that of pear, apple, tomato, banana and white grape and it is in the same order as pink grapefruit, red grape and orange. It seemed interesting that the extracts from the white cultivar, that tested very little betanins, exhibited the highest protective action of lipid oxidation in this study. Butera *et al.* (2002) concluded that red, yellow and white prickly pear fruit have a marked *in vitro* antioxidant activity in both chemical and biological systems and that betalain pigments may be responsible for this observed high antioxidant activity. This statement seems to be contrary to the findings; if all colours of cactus pear fruit exhibited high antioxidant potential levels, it could not be attributed to betalains as it predominates in red and purple fruit

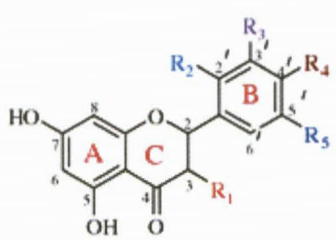
according to Stintzing *et al.* (2005); Castellanos-Santiago and Yahia (2008); Figueroa *et al.* (2010) Sumaya-Martínez *et al.* (2011) and Yahia and Mondragon-Jacobo (2011).

2.8.2 Total phenolics

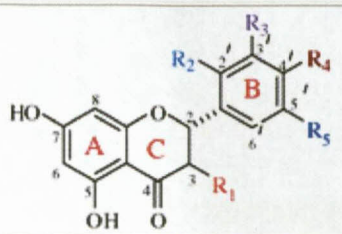
Phenolics are present in most plant materials as secondary metabolites. Infact, the majority of natural antioxidants are phenolics compounds (Gulçin. 2012). The polyphenolic family includes monomeric flavanols, flavanones, anthocyanidins, flavones and flavonols. All of these polyphenolic components have a diphenylpropane ($C_6C_3C_6$) skeleton and may act as antioxidants or as agents of other mechanisms contributing to anticarcinogenic or cardioprotection action (Rice-Evans *et al.*, 1996).

There are more than 8000 polyphenolcs, including over 4000 flavonoids that have been identified in different plant species from leaves, stems, roots, fruit and seeds. Aromatic amino acids phenylalalnine and tyrosine combine to form flavonoids therefore the basic structure is the flavan nucleus, consisting of 25 carbon atoms arraged in three rings (Table 2.3) (Gulçin, 2012).

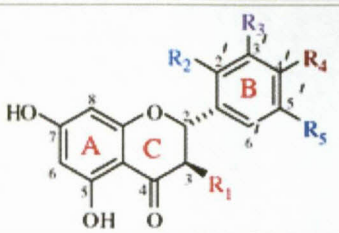
Table 2.3: Chemical structure of flavonoids



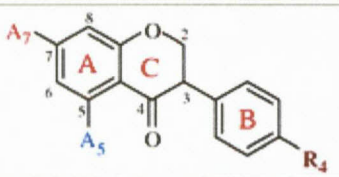
		R_1	R_2	R_3	R_4	R_5
Falavones	Apigenin	H	H	H	OH	H
	Chrysin	H	H	H	H	H
	Luteolin	H	H	OH	OH	H
Flavonols	Datiscetin	OH	H	OH	OH	H
	Quercetin	OH	H	OH	OH	H
	Myricetin	OH	H	OH	OH	OH
	Morin	OH	OH	H	OH	H
	Kaempferol	OH	H	H	OH	H



Flavanones	Hesperetin	H	H	OH	OCH_3	H
	Naringenin	H	H	H	OH	H



Flavanonol	Taxifolin	OH	H	OH	OH	H

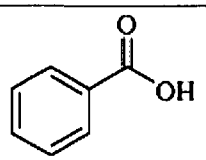
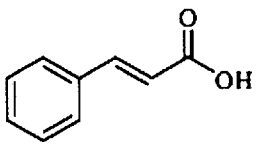


Isoflavones		**A_5**	**A_7**	**R_4**
	Genistein	OH	OH	OH
	Genistin	OH	Oglc	OH
	Daidzein	H	OH	OH
	Daidzin	H	Oglc	OH
	Biochanin A	OH	OH	OCH_3
	Formononetin	H	OH	OCH_3

(Gulçin 2012)

Phenolic acids are mostly present in bound form in plant materials and occur in esters, glycosides and other insoluble bound complexes. Phenolic acids are hydroxyl derivatives of aromatic carboxylic acids, from either a cinnamatic or benozoic acid group. The hydroxyl cinnimatic acids have been found to have significantly higher antioxidant activity than the hydroxybenzoic acids (Table 2.4) (Gulçin, 2012).

Table 2.4: Classification of phenolic acids as benzoic acid and cinnamic acid derivatives

Phenolic acids	
 (Benzoic acid derivatives)	 (Cinnamic acid derivatives)
Gallic acid	Caffeic acid
p-hydrobenzoic acid	p-coumaric acid
3,4-dihidrobenzoic acid	Rosmarinic acid
Vanillic acid	Ferulic acid
Syringic acid	Sinapic acid
Protocatechuic acid	Chlorogenic acid

(Gulçin, 2012)

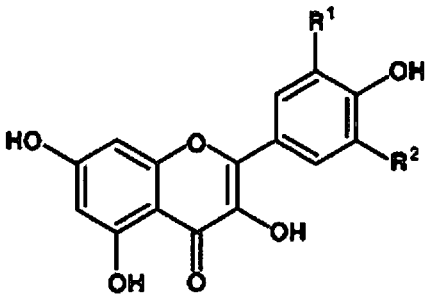
Non-chlorophyll plant pigments are usually either flavonoids or carotenoids and within in the flavonoid group, anthocyanins are usually the most important pigment, providing a range of colours from orange to red to pink to mauve to purple and blue. Anthocyanins are one class of flavonoids that are widely distributed plant polyphenols (Wrolstad, 2001). However in a certain order of plants (Caryophyllales) another class of pigments namely betalains replaces the anthocyanins. No plant has yet been found that produces both betalain and anthocyanin pigments (Harris *et al.,* 2012).

One of the more interesting facts about the phytochemical content of cactus pear fruit is that they are relatively high in flavonoids (Kuti, 2004). Flavanones and flavones are often found together in fruit such as citrus fruit, but it should be noted that there is mutual exclusion between flavones and flavonols, and anthocyanins are absent in flavanone-rich plants even though they are all part of the greater polyphenolic family (Rice-Evans *et al.,* 1995). Flavonols, flavan-3-ols, flavones, flavanones and flavanonols are classes of flavonoids that are either colourless or a pale yellow colour (Wrolstad, 2001). Therefore it may seem that the high phenolic content found in cactus pears does not contribute to its colour but does contribute greatly to its antioxidant potential.

The polyphenolic components of higher plants may act as antioxidants or as agents of other mechanisms contributing to the anticarcinogenic, anti-inflammatory, antiviral and anti-allergic activities and cardioprotective action, in fact they are multifunctional and can act as reducing

agents, hydrogen donating antioxidants and singlet oxygen quenchers. For a polyphenol to be defined as an antioxidant it adheres to two basic conditions, firstly it must retard, delay or prevent the oxidation of free radical-mediated oxidation, and secondly the resulting radical must be stable (Rice-Evans *et al.,* 1996). Many *in vitro* studies have shown the potential of polyphenols as direct radical scavengers and it is capable to enhance the resistance to oxidation of low density lipoproteins that causes coronary heart disease. All of the major polyphenolic constituents, whether these are flavonols (quercetin, kaempferol, isorhamnetin), flavones (luteolin), flavanols (catechins), or anthocyanins (cyanidin), has greater antioxidant capabilities than vitamin C, vitamin E and β-carotene (Rice-Evans *et al.,* 1996).

The presence of flavonoids in cactus pear fruit is an issue with which there have been inconsistencies in the research. Kuti (2004) found flavonoids in *Opuntia* cactus to be kaempferol, quercetin and isorhamnetin (Figure 2.3). Quercetin, a thoroughly studied flavonoid, which possesses antiproliferate, anticarcinogenic and antioxidant activities was found to be the predominant cactus pear flavonoid and correlated with the antioxidant capacity. It was suggested that the oxygen radical absorbance capacity (ORAC) in cactus fruit is poorly correlated with the ascorbic acid content but the phenolics contributed more significantly to the total antioxidant capacity of the different species of cactus pear fruit (Kuti, 2004).



Quercetin (R^1 = OH; R^2 = H)

Kaempferol (R^1 = R^2 = H)

Isorhamnetin (R^1 = OCH_3 ; R^2 = H)

Figure 2.3: *Polyphenolics in the cactus pear fruit* (Kuti, 2004)

Tesoriere *et al.* (2005) failed in a previous study to find polyphenols in betalain free extracts of yellow and white cultivars and could detect only a small peak (at 360 nm) in red cultivar fruit pulp (Butera *et al.,* 2002). In the subsequent study they did find very small amounts of kaempferol only in yellow fruit and it was declared that quercetin, rutin and isorhamnetin are

absent in Sicilian cultivars. In three cultivars that were tested before (Butera *et al.*, 2002) the samples showed a small absorbance peak in the red fruit, indicating the presence of flavonols and using quercetin as standard, an amount of 237 mg flavonols/100 g was calculated. However, it was concluded that betalain pigments play a more important role in antioxidant capacity present in cactus fruit cultivars from Sicily.

The flavonoid profile of cactus pears according to Galati *et al.* (2003) in whole fruit (pulp and peel) juice (95% yellow and 5% red coloured cultivars) was identified as isorhamnetin (0-methylated flavonol) glycocides at the highest peak, therefore isorhamnetin derivates seem to characterize prickly pear juice. The mean concentration of the total phenolic compounds was 746 µg/ml. The study clearly demonstrated that the presence of high amounts of polyphenolics could contribute to the high level of antioxidant capacity in cactus pear fruit juice. The flavonols stimulate the production of prostaglandins in isolated cells of the gastric mucosa, therefore it shows protective activity against ulcers, by promoting mucus secretion, enhancing mucosal blood flow and reducing microvascular permeability (Galati *et al.*, 2003)

Fernández-López *et al.* (2010) carried out an analytical study to find the presence of antioxidant constituents in three species of Spanish red-skinned cactus pears and found that the phenolics content ranged from 164.6 to 218.8 mg/100 g gallic acid equivalents (GAE). It was suggested that the values were high because the peel in addition to the pulp was tested and that phenolics may tend to accumulate in the dermal tissues of the plant body due to their potential role in protection against UV radiation. Phenolics in the peel would act as defense chemicals against pathogens and predators. The amount of phenolics that was found in cactus pear fruit were higher than the amount found in other popular fruit such as peaches, plums and nectarines.

It seems to be established that flavonols are present in cactus pear fruit, but there is also discrepancy in the data derived from different researchers whether polyphenols (flavonoids) occur more in purple coloured fruit or whether the phenolics found in cactus pear fruit has no relation to its colour. More studies have proven that purple or red coloured fruit contained more polyphenolics. Kuti (2004), found that the purple skinned *Opuntia lindheimeri* cactus pear variety had the highest total flavonoids (93.5 ± 12.4 µg/g) followed by the green skinned *Opuntia ficus-indica* (69.5 ± 3.8 µg/g), the red skinned *Opuntia streptacantha* (54.8 ± 5.1 µg/g) and the yellow skinned *Opuntia stricta var. stricta* (9.8 ± 3.0 µg/g). The purple fruit (*O. lindheimeri*) that had the highest polyphenol content also exhibited the highest antioxidant capacity. It was speculated

that phenolics only, or perhaps a synergistic effect between different bioactive compounds, may contribute to the exceptionally high antioxidant capacity found in cactus pear fruit (Kuti, 2004).

Stintzing *et al.* (2005) tested the juice obtained from the entire fruit including the non-edible peel portion and found that purple fruit juice had the highest concentration of total phenolics (660 ± 35.8 mg/l), in fact it was twice that of red- (335 ± 19.3 mg/l) and three times orange- (247 ± 23.1 mg/l) and green (242 ± 13.4 mg/l) fruit. These results compared to that of peach, plum and nectarine fruit.

Alimi *et al.* (2012) found 782 ± 0.94 µg/ml GAE in unpeeled purple skinned *Opuntia ficus f. inermis* prickly pear. Yahia and Mondragon-Jacobo (2011) found that "O. *ficus-indica* Naranjona" (orange) and "*O. ficus-indica* Camuesa" (purple) had the highest total phenolic contents of about 130 mg/g GAE. Chavez-Santoscoy *et al.* (2009) found a range from 22.3 to 226.3 µg/g GAE in nine different fruit of which the highest amounts were found in a cultivar that has red skin and purple pulp. Interestingly, the fruit with the lowest amount was also a purple cultivar.

Morales and Sáenz (2009) reported 777.43 mg/l GAE in the purple and 371.95 mg/l GAE in orange cactus pear fruit pulp. They found that the pulp with the highest pigment content also had the highest bioactive compounds. Coria-Cayupán *et al.* (2011) found total phenolic compounds contents of 1.1 mg/g GAE for dark purple, 0.9 mg/g GAE for purple and 0.6 mg/g GAE for green fruit of *O. ficus-indica*. Purple, dark purple and orange presented the highest levels of all the coloured fruit (dark purple, purple, pink, orange, yellow and green) and yellow exhibited the lowest levels in this study.

In the study done by Sumaya-Martinez *et al.* (2011), one of the six red cultivars studied proved to have the highest amounts of phenolics (670 and 509 mg/l GAE), while another one of the six red cultivars studied proved to have the lowest amount (138 mg/l GAE). The mean values for purple, red, white and yellow fruit were actually very similar, with some white coloured fruit showing higher values than some purple and red fruit. They concluded that the total phenolic concentration does not correlate with the colour of fruit as there were statistically significant differences among all studied cultivars of cactus pears as well as cultivars of the same colour. Furthermore, they reported that the highest concentration of total phenolics was found in cultivars of different colours, not only purple cultivars, as was reported by Kuti (2004).

Figueroa-Cares *et al.* (2010) found that white coloured fruit had significantly lower phenolic contents than all the other coloured cultivars. In fact the highest concentration of phenols was found in yellow and orange fruit and in this study, no relationship of phenols to the colour of the fruit was found.

Stintzing *et al.* (2001) speculated that the apparent relationship between phenolic content and colour within the fruit appears to depend on the species, but authors such as Galati *et al.* (2003) stressed that the microconstituents found in cactus pear depended on the processing method, the degree of ripening, the storage temperature of the juice, the cultivar and the origin. Nazareno *et al.* (2009) evaluated the changes in the bioactive substances such as polyphenolics after refrigeration for three and four weeks and found that the phenolics were preserved during the time it would take for fruit to reach markets or to be exported to Europe. Piga *et al.* (2003) found that polyphenol content declined significantly in fruit after six days of storage, but that the overall health-promoting properties did not decrease. After Morales and Sáenz (2009) produced a topping and hence exposed cactus pear fruit to heat, the phenolics showed a degradation of 54.91% in purple and 64.65% in orange toppings.

Polyphenols seems to be present and highly active in all the different parts of the cactus pear fruit plant. Ramírez-Moreno *et al.* (2011) found that the polyphenol content in the pulp was twice as high as in the seeds. They found that polyphenols in cactus pears are highly bio-accessible as only a small part of the polyphenols present in the pulp was trapped in the food matrix after digestion. A high correlation was found between polyphenol content and the antioxidant capacity, suggesting that phenolics were the major contributors. Both pulp and seeds were found to be good sources of phenolic compounds, both provided high capacity as antioxidants, and the polyphenol content is highly bio-accessible as it could reach the colon where it will contribute to the antioxidant environment.

Santos-Zea *et al.* (2011) determined the phenolic content in the cladodes of nine different varieties of *Opuntia* spp. and found very different amounts, with the lowest content at 318.1 µg/g GAE. It was stated that polyphenols in plants are highly variable and that variations may even occur within a single species. The amount found was 2-15 times lower compared to amounts found in other studies but concluded that phenolic compounds could have been affected by the disinfection of the cladodes and thus the cladodes could have been thermally damaged during the drying of cladodes to produce flour. Guevara-Figueroa *et al.* (2010) found very small amounts of phenolics and flavonoids in cladodes from Mexico. The values attained varied from

19.9 mg/g GAE for the wild O. *ficus-indica* Morado variety to 17.8 mg/g GAE for O. *ficus-indica* Cristalino. The O. *ficus-indica* Tapon (purple fruit) varieties had the lowest phenolic content with values less than 2 mg/g GAE. The researchers stressed that phenolics are lost during heat treatment therefore caution must be taken upon the drying of cladodes.

Ginestra *et al.* (2009) analysed fresh cladodes from Southern Italy and found very low phenolic components. The predominant flavonoids in the cladodes were isorhamnetin with even smaller concentrations of kaempferol or quercetin. Guevara-Figueroa *et al.* (2010) also found phenolic acids and flavonoids at very low amounts in cladodes from Mexico. Gallegos-Infante *et al.* (2009) tested cladodes from Mexico and found that the antioxidant capacity was not related to the polyphenol concentration found in cladodes. It is thus evident that low levels of phenolics were found in cladodes.

Medina-Torres *et al.* (2011) investigated dried cladodes and found that the total phenol content of samples was 1g gallic acid/kg for cladodes on a dry basis. Gallegos-Infante *et al.* (2009) found that the total phenolics in dried samples were lower than that of fresh samples (180 mg/g GAE dry weight). The lower content found in dried samples could be a result of heat during the drying process, thus a longer time of drying at a lower temperature could increase the content of the total phenolics in dried samples.

According to Bensadón *et al.* (2010) polyphenols bound to dietary fibre can account for a substantial part of total polyphenols present in food. On average, 2.5% of insoluble dietary fibre in common fruit and vegetables consists of polyphenols. High amounts of extractable phenolic compounds were found in their study of cladodes and fruit by-products of *Opuntia* varieties (from 1.54 g/100 g in red fruit variety and up to 3.71 g/100 g in cladodes).

According to Diaz Medina *et al.* (2007) there is no recommended dietary intake for total polyphenolic content. However, the American Cancer Society has established 100 mg/day of flavonoids as an adequate amount for the prevention of cancer and degenerative illness. This means that one cactus pear fruit portion would provide more than half the daily requirements in adults.

2.8.3 Ascorbic acid

Vitamin C is not only a powerful natural antioxidant but is also one of the least toxic. It is a special antioxidant because it can transfer a single electron and can successfully reduce a reactive compound such as the superoxide radical H_2O_2, the hydroxyl radical and singlet oxygen (Gülçin, 2012). Ramírez-Moreno *et al.* (2011) found that ascorbic acid in cactus pears is in the

reduced form, indicating that ascorbic acid in the pulp could largely contribute to the anti-oxidant capacity of cactus pear fruit in the human body (Figure 2.4). It was found that the short-term supplementation of 500 g fruit pulp daily for two weeks, positively affected the body's redox balance, decreased lipid oxidation and improved antioxidant status of healthy humans.

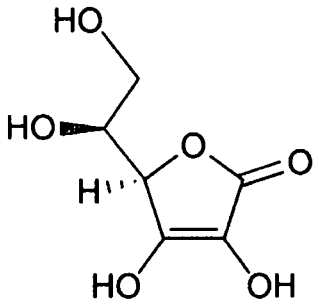


Figure 2.4: *Ascorbic acid molecule* (http://www.livingintherealworld.net/healthy/page/11/)

Major fruit sources of ascorbic acid are citrus fruit, kiwi fruit, cherries and melons. The ascorbic acid content in fruit could exceed 100 mg/g (fresh weight) such as in kiwi fruit that contain 105 mg/g ascorbic acid (Gülçin, 2012). Moβhammer *et al.* (2006a) established that cactus pears have a higher vitamin C content compared to most other fruit (apple, pear, grape and banana). El-Samahy *et al.* (2006) concluded that the high occurrence of chemical components, together with the unique characteristics and attractive colour, make the cactus pear a very suitable, natural source of energy, nutritive components and antioxidants.

Different researchers found different amounts of ascorbic acid in the fresh cactus fruit (up to 41 mg/100 g), depending on the cultivar of cactus pears, but agreed that ascorbic acid levels were generally high compared to other common fruit (Sáenz, 2000; Piga, 2004).

When it came to which colour cactus fruit had the highest levels of ascorbic acid, there was much discrepancy in the data and the findings of different researchers from different regions around the world. The most researchers reported that purple cactus fruit had the highest levels. (Figueroa-Cares *et al.* 2010; Yahia & Mondragon-Jacobo, 2011; Sumaya-Martínez *et al.,* 2011; Coria Cayapán *et al.,* 2011). Red cactus pear fruit tested the highest in the studies done by Kuti (2004) and Fernández López (2010). De Wit *et al.* (2010) found the highest ascorbic acid contents in orange coloured fruit and Stintzing *et al.* (2005) in white coloured fruit. Díaz Medina *et al.* (2007) found no difference between ascorbic acid in green and orange fruit. The measured contents of ascorbic acid varied in these studies from 0.85 to 48 mg/100 g.

In general, the fruit that contained the highest ascorbic acid levels also had the highest phenolic contents and contained fair amounts of β-carotene as well (Yahia & Mondragon-Jacobo, 2011). Díaz Medina *et al.* (2007) and Coria Cayapán *et al.* (2011) calculated that one portion of cactus pear fruit would contribute 43% of daily intake recommendations for Vitamin C as the daily recommended intake for vitamin C is 60 mg/day.

De Wit *et al.* (2010) observed that large variations are found in the quality characteristics, including the ascorbic acid contents, at different locations, in different genotypes and in fruit from different seasons. The ripening stage of the fruit could also be responsible for discrepancies findings as was found by Coria Cayapán *et al.* (2011) who found that during the ripening process (monitored over 8 weeks) the ascorbic acid content showed a steady increase for yellow as well as orange fruit. The free radical scavenging action increased during fruit ripening and this was attributed to the presence of ascorbic acid as well as other active compound (Coria Cayapán *et al.*, 2011). It is therefore questionable whether there is a relationship between ascorbic acid and cactus pear colour.

Tesoriere *et al.* (2005) determined the ascorbic acid content in cactus pear juice (not indicated which colour was tested) before pasteurization (1.75 mM) and after processing into an industrial concentrated juice (0.17 mM). It was clear from the results that vitamin C is unstable during processing even under less severe thermal treatments. Moβhammer *et al.* (2006b) found 85% to 90% decay of vitamin C during concentration of juice from O. *ficus-indica* Gialla. Nazareno *et al.* (2009) looked at the effect of refrigerated storage over 4 weeks in yellow spineless cactus pears. The ascorbic acid content only slightly decreased (0.29 to 0.28 mg/g) which indicates that no important vitamin losses were detected. These results are consistent with the behaviour expected for non-climacteric fruit such as cactus pear fruit.

Piga *et al.* (2003) observed the changes that take place at 0, 3, 6 and 9 days of minimally processed film packaged fresh cactus pear fruit during cold storage. It was found that ascorbic acid showed no significant variations (1.82, 1.78, 1.67 and 1.85 mg/g respectively). The researchers would have expected a greater decrease but theorized that ascorbic acid was probably protected by the ascorbate-sparing effect of polyphenols. They concluded this as the polyphenols significantly declined after six days of storage.

Stintzing and Carle (2005) reported that the total vitamin C in 100 g of fresh cladode was between 7 and 22 mg in *Opuntia* spp. Medina-Torres *et al.* (2011) determined ascorbic acid content of fresh and dried cladodes, in fresh cladodes it was 2.05 g/kg. It was found in this study

that there was a big loss in the 45 °C and 65 °C dehydrated samples as compared to the fresh cladodes. The loss of more than 80% between the fresh and dried samples was attributed to the high temperature used during the drying of the samples.

2.8.4 Carotenoids

Carotenoids are lipophilic, natural pigments that are responsible for red, orange and yellow colours in plants and in some birds, insects and fish (Stahl & Sies, 2003). The pattern of double bonds that is conjugated in the polyene backbone influences the antioxidant capacity of carotenoids. Depending on the number of double bonds and cis/trans configurations, several carotenoid molecule types may be found in fruit, of which β-carotene and lycopene are the most common (Figure 2.5). A mixture of mono- and poly – cis-isomers form during isomerization in addition to the formation of the all-trans form, which is the predominant form. Carotenoids are most likely involved in the scavenging of the reactive oxygen species, singlet molecular oxygen and peroxyl radicals. The energy of singlet molecular oxygen is transferred to the carotenoid molecule to yield ground state oxygen and triplet excited carotene (Stahl & Sies, 2003). The carotenoid then returns to ground state dissipating the energy and can be re-used in these energy-quenching cycles. Vitamins E, C and β-carotene (Vitamin A) are co-operative and synergistic in their work as scavengers of reactive radicals. Carotenoids are therefore efficient antioxidants that protect plants, animals and humans against oxidative damage (Stahl & Sies, 2003).

Feugang *et al.* (2006) stated that fat soluble β-carotene is found in the lipid fraction of the cactus pear fruit, namely in the seeds and pulp. As it is predominant in the pulp lipids, it improves the stability of fatty oils through its antioxidative properties. Kuti (2004) have shown that high intakes of carotenoid rich vegetables and fruit that lead to high blood levels of β-carotene are associated with decreased incidence of some cancers.

The data from all research into carotene content in cactus pear fruit suggests that there are very small amounts present. As was the case with the other studied antioxidants, there remains great controversy as to which colour cactus pear fruit contains the most carotenoids. Kuti (2004) found that the yellow skinned cactus pears had the highest total carotenoid concentrations, that ranged from 6.0 to 17.7 µg/g (fresh weight) while the green skinned varieties had the lowest carotenoid concentrations, which ranged from 1.2 to 1.7 µg/g fresh weight, respectively. This data suggests that the yellow skinned fruit usually have higher carotenoids than other coloured cactus pear fruit. Figueroa-Cares *et al.* (2010) found that the relationship between pulp colour

and the presence of chlorophyll and carotene is indirect as yellow and white cactus pears showed the highest chlorophyll content but also the lowest carotene content.

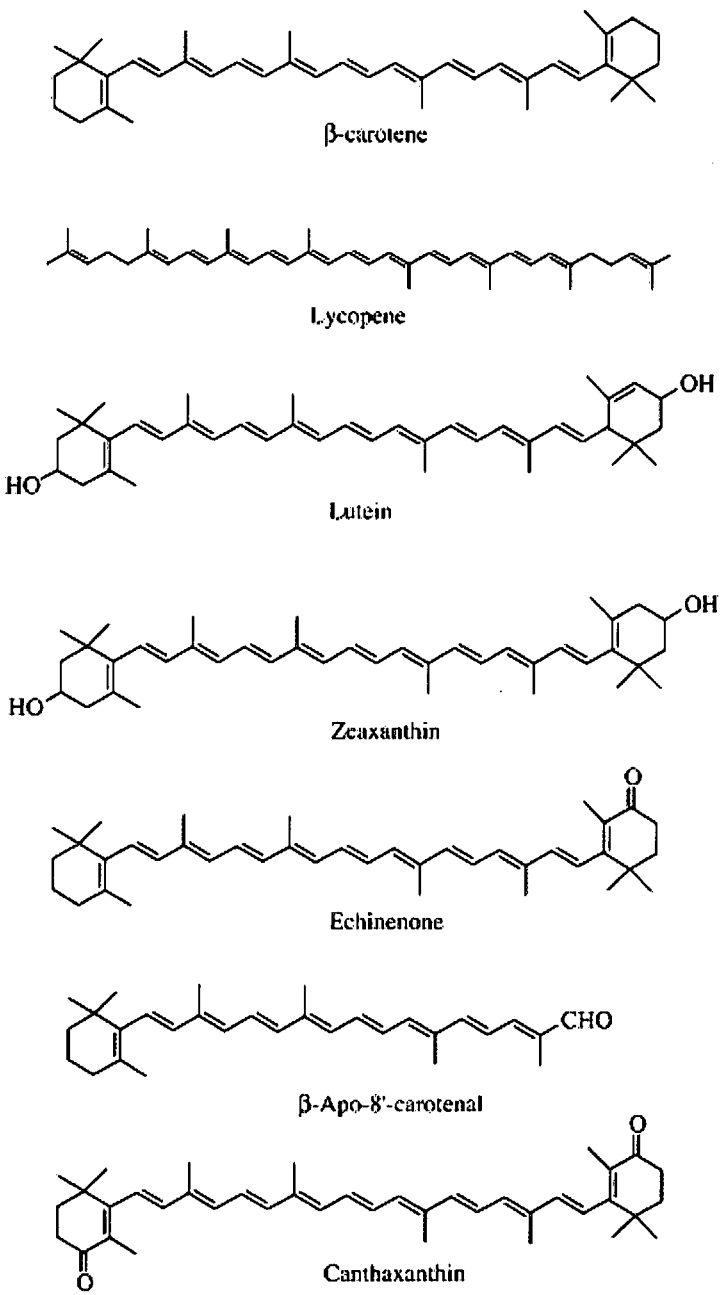


Figure 2.5: *Carotene molecules in fruit* (Dufossé *et al.*, 2005)

When Yahia and Mondragon-Jacobo (2011) determined the carotene content in 10 ten cultivars from Mexico, they found that fruit from all the cultivars (purple, red, orange, yellow and white) had low levels of total carotenoids. In contrast to Kuti (2004) who found that the yellow skinned cactus pears had the highest total carotenoid concentrations, it was found that Camuesa (purple coloured fruit) had the highest at 2.5 µg/g dry weight and pink-red coloured fruit the lowest at 1 µg/g dry weight. According to their data, the cultivar with the highest betalains in their study also had the highest amount of carotenoids. It was speculated that the fruit with high β-carotene content would normally present as yellow coloured fruit but the betalains (purple) mask the yellow-orange colour of β-carotene, therefore purple fruit actually have the highest β-carotene concentrations but don't appear to do so.

Tesoriere *et al.* (2005), Alimi *et al.* (2010), Morales and Sáenz, (2009), Stintzing and Carle (2005), Figueroa-Cares *et al.* (2010) and Fernández-López *et al.* (2010) measured carotene in yellow, red and white fruit from Sicily, Spain, Tunisia, Chile and Mexico and found very modest amounts of carotenoids in all cultivars with purple and red fruit measuring the highest (3.47 µg/100 g) and the lowest in white fruit. Therefore cactus pear does not seem to be a good source of carotenoids in comparison to other fruit such as mangoes and cantaloupe and vegetables such as spinach and broccoli. Mainly lutein (0.044 µg/g) and β-carotene (1.85 µg/g) was found. It was speculated that low amounts of carotene could contribute to antioxidant properties even in small amounts. Figueroa-Cares *et al.* (2010) concluded that their results show that the relationship between fruit colour and presence of carotene is indirect; therefore colour is a poor indicator of carotene content in cactus pear fruit.

Hernández-Pérez *et al.* (2005) looked at the nutritional changes in three varieties of Mexican cactus fruit as they ripen and found that the carotene content changed slightly every time it was tested at 7, 14 and 28 days. There was no consistent trend in changes seen. The carotene content was consistently low with 0.82 mg/g (fresh weight) being the highest measurement at 0 and 7 days for *O. ficus-indica* "Naranjona".

Processing diminishes carotene content in fruit as Morales and Sáenz, (2009) found that a degradation of carotenoids took place during the preparation of a topping. The purple topping showed 90.70% degradation and the degradation in orange was 97.87%. Tesoriere *et al.* (2005) found the same phenomenon as after processing cactus pear fruit into a concentrated juice, the carotenoids were lost (not detected) as the result of thermal degradation during pasteurization and concentration.

Medina-Torres *et al.* (2011) determined total carotene for fresh and dried cladode samples and it was found that fresh nopal contained 1.16 g/kg, but when dried at 45°C, only 0.543 g/kg remained. This means that the β-carotene content was approximately 50% less than that of the fresh sample. The data suggested that fresh cladodes have a higher content of carotene than that found in other vegetables such as baby carrot, beetroot, spinach and lettuce. Bensadón *et al.* (2010) used two varieties of young cladodes from Mexico and found the carotenoid content were quite high at 21.32 mg/g and 22.84 mg/g of dry matter. In both the cladodes and fruit by-products (seeds), there were no significant differences in the carotenoid content between the cladodes from different coloured fruit varieties. Stintzing and Carle (2005) reported that the carotenoid profile of fresh cladodes revealed α-cryptoxanthin (20%), β-carotene (36%) and lutein (44%) content.

Jaramillo-Flores *et al.* (2003) tested cladodes from Mexico and identified three types of carotenoids in cladodes. The first had an orange colour (β-carotene), second had a light yellow colour (cryptoxanthin) and the third was lutein. The total carotenoid content was 231.8 µg/g on a dry basis which corresponds to 36% β-carotene, 46% lutein and 18% cryptoxanthin. They stated that the higher the temperature, the more extractable the carotenoids. In the case of cladodes, the nature of the carbohydrates is such that it may form complexes with the carotenoids. In this case a thermal process can increase its bioavailability therefore cooking may allow a maximum retention of carotene. Cladodes from Mexico only had β-carotene concentrations in fresh cladodes at 1.16 g/kg. For dehydrated samples a loss of approximately 50% occurred. The β-carotene content was higher than that found in baby carrot, beetroot, spinach and lettuce (Medina-Torres *et al.,* 2011).

From the literature the conclusion could be made that fruit (pulp) contain very modest amounts of carotene but that the cladodes and seeds may be good sources. No data on the peel alone has been published.

2.9 Antioxidant activity and -capacity of fresh fruit and cladodes

Oxidation takes place when electrons are transferred from one atom to another. It represents a vital part of aerobic life and in metabolism. Oxygen is the ultimate electron acceptor in the electron flow system and ATP is formed by the energy that is produced but it is when electrons become uncoupled that free radicals are generated. With other words the transfer of unpaired or single electrons creates free radicals known as reactive non radicals (ROS). Free radicals are

very reactive; it attacks and damages the molecules in any nearby cells in the body (Gülçin, 2012).

"A free radical is defined as a chemical species capable of independent existence, processing one or more unpaired electrons" (Gülçin, 2012). ROS are produced in the body during normal activities such as immune defense and are even required for certain cell functions. It is only when it accumulates that it damages nucleic acids, lipids, proteins, carbohydrates and polyunsaturated fatty acids. In certain instances it may cause DNA damage and stimulate chain reactions that lead to ageing, cancer and many other serious diseases (Gülçin, 2012). ROS can also enter the body from external sources such as tobacco smoke, pollutants, organic solvents and pesticides. The diet may also include ROS. UV light can cause a reaction that causes highly reactive species and an important signaling molecule in the body may become cytotoxic when it is present in excessive amounts (Gülçin, 2012).

A healthy cell has enough antioxidant defenses against ROS but the delicate balance is shifted when pro-oxidants increase or the antioxidants are diminished; that is when the cell goes into oxidative stress. Oxidative stess is defined as "the dissolution of the pro-oxidant-antioxidant equilibrium". Therefore the body's natural ability to defend itself and detoxify the reactive compounds or to repair itself disappears. This lowering of the antioxidant levels in the body can be due to mutated antioxidant, toxins, or the reduced intake of antioxidants through the diet (Gülçin, 2012).

An antioxidant is a molecule that is able to reduce, delay or inhibit oxidation of other molecules even when present in very low levels. It therefore protects the body against diseases. Antioxidant activity in food refers to the rate (speed) of a reaction between an antioxidant and an oxidant. Antioxidant capacity is an amount (measure) of a free radical scavenged and destroyed by the antioxidant. Vitamin C, E and A are the most well known antioxidants in the human diet. There are many other lesser known but not less important antioxidants and together they are believed to act synergistically. Fruit and vegetables have high levels of antioxidants and therefore it is associated with health and reduced risk of chronic diseases. There is a growing interest among the public for safer, healthier food products and a growing trend of consumers who prefer natural foods and additives such as antioxidants, pigments and preservatives (Gülçin, 2012).

Multiple procedures exist and could be used to measure antioxidant properties of food components that reflect their potential protective effects (Gülçin, 2012).There are different

opinions on the appropriate method of accessing antioxidant capacity in plant tissues; therefore most studies use at least two different assays to measure the potential of antioxidants (Butera *et al.,* 2002). The tests (assays) are often standardized with a synthetic antioxidant (Trolox) and results may be expressed as Trolox equivalents (TE) per gram or liter (Butera *et al.,* 2002). Trolox equivalence antioxidant capacity (TEAC) tests directly determine the radical scavenging capacity of a compound by reducing the 2, 2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABST) radical cation, the oxygen radical absorbance capacity (ORAC) assay monitors the inhibition percentage and time of oxidation (Stintzing *et al.,* 2005) by measuring the inhibition of peroxyl radical-induced oxidations (Gülçin, 2012).

2.9.1 DPPH radical scavenging test

The 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay relies on the principle that radical scavengers may directly react with and scavenge peroxide radicals in order to terminate the peroxidation chain reactions and improve the quality and stability of food products (Gülçin, 2012). It is the oldest indirect method for determining antioxidant activity and is a simple and rapid method that is used frequently in antioxidant screening tests.

2.9.2 Chelating ability of ferrous irons

In the light of using different methods of determining antioxidant potential, the metal-chelating assay is a fair choice. Iron is an essential mineral in the body but excess can result in injury to cells. Iron may undergo a reaction that reduces it forming a highly reactive hydroxyl radical and thereby contributing to oxidative stress. These radicals cause damage to cellular lipids, nuclei acids, proteins and carbohydrates. Ferrous ions (Fe^{2+}) are the most effective pro-oxidants in the food system and have good chelating effects that remove free iron ions from circulation. Therefore minimizing ferrous ion is protective against oxidative stress and an effective ferrous ion chelator could offer this protection by removing iron that may otherwise participate in $HO^{\cdot}$ generation. The chelating ability of antioxidants may be an alternative but effective way of preventing oxidative stress and therefore several diseases (Gülçin, 2012).

It is evident from the literature that although there are different findings and opinions as to which antioxidant and colour provides the highest antioxidant capacity, it is agreed amongst researchers that cactus pear fruit improve the redox status in humans, suggesting major benefits to health. Cactus pear juices contained at least twice the antioxidant capacity of strawberry, plum, orange, grapefruit, red and white grape, kiwifruit, apple, pear and tomato and

were more comparable to red wine and pomegranate, concord grape, blueberry and black berry juices (Seeram *et al.*, 2008; Yahia & Mondragon-Jacobo, 2011). They concluded that although different coloured fruit have different antioxidant levels, they all had very high antioxidant capacity and thus important nutritional and health advantages. Diets that include cactus pears could help to reduce the risk of age-related and degenerative diseases in which the level of body oxidative stress play a pathogenic role. Although more studies on humans on a large scale are required, cactus pear fruit appeared to be a functional food (Livrea & Tesoriere, 2009).

As far as the contribution of ascorbic acid toward antioxidant capacity is concerned, there seems to be agreement that ascorbic acid is not the reason for the high antioxidant capacity found in cactus pear fruit. In earlier research done by Wang *et al.* (1996), it was reported that antioxidant capacity is poorly correlated with ascorbic acid content in all types of fruit, thus suggesting that other components such as phenolics (flavonoids) contribute more significantly to the total antioxidant capacity. It is known that prickly pear fruit are a very good source of vitamin C, but it may only account for 30-40% of the total antioxidant activity that the fruit demonstrates.

In the study by Stintzing *et al.* (2005) on ten different cultivars, 9 of *O. ficus-indica* and one of O. *robusta* from the experimental fields in California it was determined that the total phenolics dominated the contribution to the total antioxidant capacity since a good correlation between phenolics, TEAC and ORAC values were seen. The contribution by betalains was much higher than that provided by ascorbic acid; in fact the contribution by ascorbic acid was very small. In other words the highest correlation was found for total phenolics, then betacyanin and betaxanthin and lastly ascorbic acid. These findings can be accepted when considering the data concerning betalains in green coloured fruit: it contains very little betalains, yet it has substantial antioxidant capacity therefore indicating the colourless phenolic compounds as the largest contributors to antioxidant capacity. Table 2.5 is a summary of the antioxidant content and the corresponding capacity in pure cactus pear juice as well as the edible pulp as found by Stintzing *et al.* (2005).

It was concluded in a study by Kuti (2004) that cactus pear fruit was a rich source of antioxidants. The findings were in agreement with that of Stintzing *et al.* (2005) that phenolics may be the highest contributor to the antioxidant value. In this study into four *Opuntia* species (green-skinned *O. ficus-indica,* red-skinned *O. streptacantha*, yellow-skinned *O. stricta* and purple-skinned *O. linderheimeri*) from the United States of America, data showed that the antioxidant capacity in terms of Trolox ranged from low (15.8 µM Trolox equivalence (TE)/g) in

the yellow skinned fruit to high (49.2 µM TE/g) in the purple skinned fruit. Although the data suggested that antioxidant capacity and flavonoid contents were significantly different among varieties (colour) of cactus pear fruit, there was a correlation between flavonoid content and antioxidant capacity. No correlation between antioxidant capacity and ascorbic acid or carotenoid content in cactus pear fruit was observed. The purple-skinned cactus pears had the highest flavonoid as well as antioxidant potential and the yellow-skinned fruit had the lowest flavonoid as well as antioxidant potential. Therefore the observation was made that high antioxidant capacity in purple-skinned cactus pear, observed in this study may be due to high phenolic contents or a combination of individual antioxidants producing a synergistic effect. Although red-skinned fruit contained the most ascorbic acid (815 µg/g fresh weight) and yellow-skinned fruit the most carotenoid (12.7 µg/g fresh weight), the antioxidant activity of the purple-skinned fruit were higher than the other varieties. This was found to be only consistent with total flavonoid content of the purple skinned fruits (Kuti, 2004).

Another study by Sumaya-Martínez *et al.* (2011) confirmed the findings by Stintzing *et al.* (2005) and Kuti, (2004). It was stated in this study that average antiradical activities of red and purple cultivars were higher than that of yellow and white fruit. The red fruit had the highest values (around 12 000 TE µmol/l) and showed antioxidant activity of over nine times that of white, yellow and other red cultivars (Sumaya-Martínez *et al.,* 2011). The antiradical activity for red cactus pears was correlated to the concentration of phenolics and ascorbic acid.

Table 2.5: Total Phenolics, Betaxanthins, Betacyanins and Ascorbic Acid contents and corresponding TEAC and ORAC Values (Fluorescein-Based) in pure cactus juice and edible pulp (January Fruit).

Cultivar	Brix	pH	Total Phenolics	Betaxanthins	Betacyanins	Ascorbic acid	TEAC		ORAC hydrophilic		ORAC lipophilic
							juice	pulp	juice	pulp	
	(°Bx)		[mg/l]	[mg/l]	[mg/l]	[mg/l]	[mmol/l]	[mmol/kg]	[mmol/l]	[mmol/kg]	[mmol/l]
Green	14.2	6.5	242	0.4	0.1	51.1	3.31	2.24	5.45	3.68	0
Orange	14.6	6.3	247	76	6.6	70.2	3.1	2.32	5.83	4.36	0
Red	14.8	5.6	335	67	120	67.9	3.71	2.6	6.35	4.44	0
Purple	12.8	6.3	660	19	431	95.4	4.99	3.64	11.2	8.16	0

Data for cactus pear pulp were calculated from the values obtained for juices considering the respective conversion factors mentioned above (sample preparation).
Values expressed as means of duplicate determinations
Total phenolis as Gallic acid equivalents
Betaxanthins as indicaxanthin equivalents
Betacyanins as betanin equivalents
(Stintzing *et al.*, 2005)

When the same researchers repeated the test using the β-carotene and linoleic acid method (where the juices were evaluated according to their protective effect on the discoloration of a β-carotene-linoleic acid emulsion), there were no significant differences between the purple, red, yellow and white cactus pear cultivars; in fact the different coloured fruit yielded similar values. They attributed this to the reaction of the media, being an oily medium. Yet another method that tested the chelating activity of ferrous ions in the juice was performed. It was concluded that chelating activity does not depend on the colour of the cactus pear. It is noteworthy that one purple variety showed the lowest and another purple variety the highest percentage of chelating activity. The different cactus pears measured activity levels that did not significantly differ ($p < 0.05$). This is the only study that reported chelating activity in cactus pears. It was concluded in the research that both the red cactus cultivars from one location (Zacatecas: *O. ficus indica* Lirio and *O. ficus indica* Lisa) showed the highest total phenolic concentrations, ascorbic acid as well as the highest antioxidant activity and free radical scavenging activity. A final conclusion was reached by Sumaya-Martinez *et al.* (2011) that red cactus cultivars showed the highest concentrations of total phenolic compounds, ascorbic acid as well as the highest antioxidant activity since the free radical scavenging activity for red cactus pears was correlated to the concentration of total phenolics and ascorbic acid. The study proved that cactus pear fruit have high antioxidant activity and may act as a powerful natural antioxidant that could be used as an antioxidant in functional foods (Sumaya-Martínez *et al.*, 2011).

Further studies that confirmed the findings by Stintzing *et al.* (2005) that phenolics may be the greatest contributor to antioxidant capacity were Fernández-López *et al.*, (2010) who found that cactus pear fruit with the highest levels of total phenolics show the highest free radical scavenging capacity thus came to the same conclusion as the researchers above. But it was found that ascorbic acid could be taken into account for overall bioactivity (up to 68%), but betalains and carotenoids were poorly correlated with antioxidant activity in these tests. Ramínez-Moreno *et al.*, (2011) found no correlation between ascorbic acid and antioxidant activity. In stead, phenolics were also suggested to be the major contributors to the antioxidant capacity of cactus pears as both pulp and seeds were good sources of total phenolics. Chang *et al.* (2008) suggested that phenolic acid and flavonoids play an important role in antioxidant activity in seeds and not ascorbic acids or betanin. In stead it was suggested that ascorbic acid and betanin may play a more pronounced role in pulp and peel.

The findings by Tesoriere *et al.*, (2004) were not consistent with Stintzing *et al.* (2005) as their data showed that betalains may be responsible for the antioxidant capacity that the fruit

demonstrates. It was apparent that something other than the vitamin C in the cactus pear fruit helped to decrease lipid oxidation and as they stated that polyphenols does not occur in Sicilian cactus pears (according to research done by Butera *et al.,* 2002), betalains must be responsible for the antioxidant capacity that cactus fruit demonstrate (Livrea & Tesoriere, 2009).

Most researchers seem to agree that red or purple fruit contribute the most toward antioxidant activity, except for Butera *et al.* (2002) who tested yellow, red and white Sicilian cultivars; they found that yellow fruit had significantly higher radical-scavenging ability than red and white ones. The white cultivar, in contrast to other studies, had the most effective lipid inhibiting oxidation ability as well as the most effective oxidation resistance. All three cultivars had quite high antioxidant capacity values and results suggested the presence of very effective electron and H-atom donors. Although the ascorbic acid levels were high, the contribution to antioxidant activity was found to be only 30 – 40%. It was speculated that betalains play a significant role in preventing lipid oxidation notwithstanding that in their data the white cultivar that virtually lacked betanin, it had the highest protective action in all models of lipid oxidation. Nevertheless, the authors suggested that betalain pigments may be important for antioxidant activity in cactus pears, as the antioxidant potential measured to more than twice that of pear, apple, tomato, banana and white grape and compared to the antioxidant levels of pink grapefruit, red grape and orange.

Yahia and Mondragon-Jacobo (2011) found that the antioxidant capacity of the ten different cultivars did not present a constant pattern with the levels of antioxidants in the different coloured cultivars. For example, one of the purple cultivars "Camuesa" tested high above the other 9 cultivars in the individual antioxidant content tests but did not prove to have the highest antioxidant capacity. It was concluded that the betalains, vitamin C and phenolics contributed more to a high antioxidant capacity than carotenoids did. Figueroa-Cares *et al.* (2010) also found that colour was not associated with the antioxidant capacity of the fruit.

Chavez-Santoscoy *et al.* (2009) used a different approach and tested the *in vitro* cancer cell viability of the juice from nine different types of cactus pear fruit species. It was found that among cancer lines tested, the viability of prostate and colon cancer cells were the most damaged. In fact, the research showed few differences among the different cactus pear fruit juices in terms of the properties and the phytochemicals. All the different cactus fruit juices had antioxidant capacity values in the narrow range of 17 to 25 TE µmol/ ml. This was despite significant variances in the contents of total phenolics, flavonoids and betalains. In comparison

to other fruit juices, the cactus pear juice had twice the capacity values of strawberry, white grape and grapefruit and was in the same range as red wine, pomegranate and black cherry. *O. rastrero* Rastrero (a spineless fruit with purple peel and pulp) had the highest antioxidant capacity (25.8 TE mmol/l) but *O. violaceae* Moradillo (a spiny fruit with purple peel and purple-red pulp) tested lower capacity levels (24.8 TE mmol/l) but had the best in vitro effect on diminishing cancer cell viability, particularly in prostate cells. The Moradillo juice also diminished the growth of normal fibroblasts that were used in the control tests. The study proved that prickly pear fruit juice could prevent oxidative stress and thus cancer. The research showed that all species of cactus pear fruit could potentially be important sources of natural antioxidants that could diminish the growth of cancer cells.

Santos-Zea *et al.* (2011) found different levels of phenols and antioxidant capacity amongst different cultivars, but cladodes from one of the cultivars were comparable with the ORAC value of cranberries and the rest to blackberries and raspberries. After processing the cladodes into flour the phenolic levels were low, as it was lost during processing but the antioxidant capacity did not diminish in the flour.

2.10 Antioxidant capacity in processed cactus fruit and cladode products

Cactus pear fruit are sweet and delicious and healthy, but the low acid content makes it very susceptible to microbial invasion. Processing is necessary to ensure that the fruit and cladodes can be preserved and used throughout the year (Joubert, 1993). Minimally processing fruit by covering peeled fruit with a high gas permeability barrier film did not decrease the ascorbic acid and polyphenols and antioxidant capacity of the fruit when stored in a refrigerator for up to 9 days (Piga *et al.,* 2003).

Most studies concentrate on the retaining of pigments after processing instead of antioxidant content and capacity but since pigmentation is contributed by antioxidants these studies are relevant. The betalains showed degradation as an L* decrease associated with a green hue was observed after one month of refrigerated storage. After heat treatments the L* decrease was ascribed to ascorbic acid degradation and hydrolytic betalain loss in the juice. It was observed that storage in light conditions promoted both betaxanthin and betacyanin degradation (Moβhammer *et al.,* 2006b). It was found by Merin *et al.*, 1987 and confirmed in studies by Lee *et al.* (2000), Son and Lee (2004) and Moβhammer *et al.* (2006b) (cited in Moβhammer *et al.,* 2006a) that colour degradation increased at higher temperatures and decreased with higher

concentrations. It seems that betaxanthins were more stable after heat treatment at pH 6 and betacyanins at pH 4. Coşkuner *et al.* (2000) initially found that the pH values affect the degree at which thermal treatment decreased cactus fruit pigment and found the optimal acidity to be at pH 5.

It is expected that processing would damage and thus decrease antioxidant content but this seems not the case with carotene. Jaramillo-Flores *et al.* (2012) studied cladodes from Mexico and found that thermally treated samples had a higher antioxidant activity than fresh samples. It was determined that the carotene extractability increased as the application of temperature treatment increased and thus the antioxidant activity increased. It was theorized that the effect that more carotene was measured after processing the cladodes, was because mucilage might interfere with antioxidant detection since the pigments can form complexes with the mucilage or pectin. It is possible that esterification of pectins could interfere with measurements before heat is applied during processing. In fact, with carotene, the higher the temperature of the thermal treatment, the higher the extractability of the carotenoids became. Another explanation for the increased measurements in processed products may be that tissue softening and morphological changes allows higher penetration of the organic solvents into cells and extracts more pigment. Rickman *et al.* (2007) studied the alledged increase in carotenoids after cooking and found that several authors reported such an increase during cooking or fresh and frozen vegetables (carrots, broccoli, green beans, spinach and peas) on a wet basis. It was also seen that frozen and cooked canned products contained equal values to fresh products, regardless of storage time. It was concluded that vegetables that were initially good sources of carotene remained good sources after processing. The same phenomenon was found by Howard *et al.* (1999) when microwave cooking caused greater solvent extraction of β-carotene in broccoli and carrots. Therefore it was concluded that carotenoids had the strongest influence on the antioxidant activity in processed cladodes as total phenolics decreased when heat was applied.

2.11 Summary

Substantial information has been made available by numerous researchers locally and internationally with regards to cactus pear plants and the health benefits that it possesses. These studies have revealed the nutritional composition of the cactus pear, bringing to light the great potential that it holds for the food industry. The presence of antioxidants in these plants have been looked into by researchers, who have found high levels in the fruit pulp, peel, seeds and cladodes. It was found that the different colours of the pulp and peel are a result of the different antioxidant dominance and thus the presence or absence of certain compounds. The potential of the antioxidants to scavenge free radicals have also been investigated and it was concluded that although different coloured fruit have different antioxidant levels, they all had very high antioxidant capacity and thus important nutritional and health advantages.

From the literature it could not be established which colour fruit, species, variety or part of the plant predominantly contained which antioxidant. Neither could it be established which of the antioxidants contributed the most towards antioxidant capacity. Yet, the excellent antioxidant potential that exists was never disputed. It is therefore necessary to investigate the South African cultivars in order to determine the antioxidant content and -potential that it may yield.

Fresh fruit and cladodes are not available throughout the year and it needs to be processed, but it is not clear from available research whether the processed products contain acceptable levels of antioxidants as research data concerning processed cactus pear fruit, peel, seeds and cladodes are very scarce. It does seem from the few investigations that do exist that processed products may remain good sources of antioxidants. In fact, antioxidants may be more available in heat treated processed products than the fresh counterparts (Jaramillo-Flores *et al.*, 2003).

The greater purpose is to educate the public in the health promoting properties of the plant in order for a greater market to be established. If the need for the fruit and cladodes in the market could be increased, rural people, farmers and even the back yard gardeners could become suppliers. The cactus pear plant could therefore become an economically viable crop if demand increased. A strong demand in the food industry for the colourants that cactus fruit contains should be encouraged as few colourants can be obtained from natural sources that are endowed with antioxidant properties that are beneficial to health.

The cactus pear plant as a crop is sustainable in South Africa as it thrives in arid and semi-arid areas that cover a large part of the country. It could be developed into a profitable industry if

South Africans would be made aware of the benefits that it holds and taught how to utilize it as a resource in their daily lives. Overall though, cactus pears are already available for commercial exploitation but the main limitation of broader commercialization is the current market prices and the lack of know-how in cactus pear processing (Moβhammer *et al.,* 2006b).

2.12 Objectives

The aim of this study was to investigate the presence and potential of antioxidants in *Opuntia ficus-indica and Opuntia robusta* cultivars found in South-Africa, not only in the raw state but also in processed products.

The fresh and processed fruit and cladodes of different coloured cultivars were analyzed for total phenolics (according to the Folin- Ciocalteu method), betalains (spectrophtometric method), ascorbic acid (2.6 dichlorophenol indiphenol titration) and carotenoids (spectrophotometric method). The study was concluded by analysis of the antioxidant capacity of the various antioxidants by measuring the free radical scavenging activity with DPPH (2, 2' – diphenyl-1-picrylhydrazyl radical) and by the chelating activity of ferrous ions.

The relationship between the ascorbic acid-, total phenolic-, betalain- as well as carotenoid content and their respective antioxidant capacities were correlated for the different parts of the fresh cactus pear plant, that is, the fruit pulp, cladodes and the by-products, namely the seeds and peel that are normally discarded as waste.

Furthermore, marketable processed products from the different parts of the plant were investigated in the same way, to compare the presence and action of above mentioned antioxidants to the fresh products.

Chapter 3

Antioxidant content and -potential of fresh fruit (pulp, peel and seeds) and cladodes from eight different cultivars of cactus pears.

3.1 Introduction

The cactus pear plant is one of Mexico's most valued resources as it has the widest germplasm variability as well as the highest number of uses. It grows and thrives in other parts of the world such as Australia, Africa, Asia and the Mediterranean basin. The cactus pear plant has received a surge of research interest as it grows mainly in arid and semi-arid regions due to its efficient use of water. It is seen as an emergent fruit crop in countries like Morocco, Tunisia, Ethiopia, Yemen and Turkey where the fruit has become more popular than other common fruit such as oranges or bananas (Yahia and Mondragon-Jacobo, 2011). The commercial value of cactus fruit can be increased in domestic as well as foreign markets if it could be distinguished from other available fruit products not only as a fresh fruit product but also as a high-value ingredient in the food industry. The unique characteristic and competitive advantage of cactus fruit could be its high antioxidant properties. It could lead to the commercialization of cactus fruit products and turn the cultivation, processing and development of new products into new business opportunities (Sumaya-Martínez *et al.*, 2011).

Antioxidants are necessary in the human body to scavenge free radicals in order to prevent a radical chain reaction of oxidation and therefore preventing the oxidation process. It is important that free radicals are stabilized as the damage that they cause in the body leads to ageing, cancer, heart disease, stroke, arteriosclerosis, diabetes and more than a hundred other diseases (Gülçin, 2012). The consumption of cactus fruit or its products may contribute substantially to the amount of antioxidants in the diet (Kuti, 2004).

The results of studies done by Sumaya-Martínez *et al.* (2011) indicated that cactus pear cultivars (specifically red and purple cultivars) show very high antioxidant activity and that the cactus fruit juice may act as a powerful antioxidant source and could be an important additive in functional foods. Kuti (2004) proved that cactus fruit has potential value as a good source of

natural antioxidants and that the antioxidant capacity is attributed to its polyphenol, ascorbic acid and carotenoid contents. Stintzing *et al.* (2005) found the highest correlation between antioxidant content and -capacity was with the total phenolic content, then betacyanins and betaxanthin and lastly ascorbic acid.

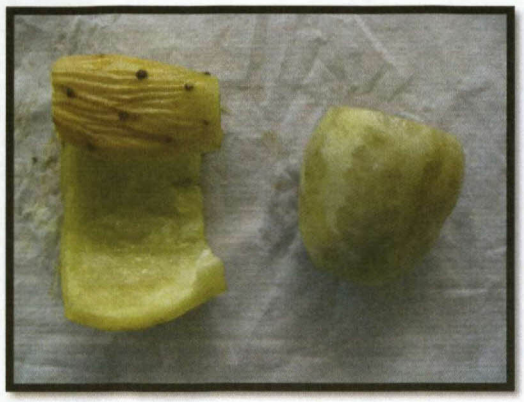
In this study the relationship between the content of antioxidants namely ascorbic acid, polyphenolics, betalains and carotenoids and their ability to prevent oxidation are investigated in a selection of different coloured cactus fruits in order to have a better understanding of the relationship between colour, antioxidant content and antioxidant potential.

3.2 Materials and methods

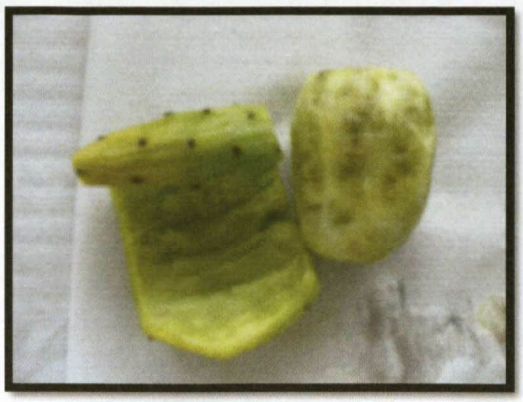
3.2.1 Fruit collection

Waterkloof is an eight year old cactus pear orchard in the Free State, located in the Bloemfontein district. It is 1.348 m above sea level and receives 556 mm rainfall on average. The GPS coordinates are 29°10'53" S, 25°58'38" E. It hosts 40 *Opuntia ficus-indica* varieties and two *O. robusta* cultivars laid out in a fully randomized design with two replications of each cultivar (De Wit *et al.*, 2010).

Ripe fruit from seven *Opuntia ficus-indica* cultivars and one *Opuntia robusta* were collected at 50% colour break stage (skin colouring) to ensure even ripeness and colour development. In January 2011, when the cactus pears ripened in the hot and dry climate of the Free State province, samples were transported to the laboratory, weighed, peeled and frozen at -18°C (not longer than two months). The eight cultivars were chosen for their colour and quality according to results of a study done previously by De Wit *et al.* (2010). Two cultivars from each of the four colours of fruit cultivated in South Africa were selected. These include two green fruit varieties from *Opuntia ficus-indica* (Nepgen and Morado), two orange fruit varieties from *Opuntia ficus-indica* (Ofer and Gymno Carpo), two red-pink fruit varieties from *Opuntia ficus-indica* (Meyers and Sicilian Indian Fig) and two red-purple fruit from two different species namely Nudosa from *Opuntia ficus-indica* and Robusta from *Opuntia robusta*. Only one purple fruit was representative of *O. robusta* sp. while the other seven cultivars were from *O. ficus-indica*. (Figure 3.1)



Green: *O. ficus-indica* Nepgen



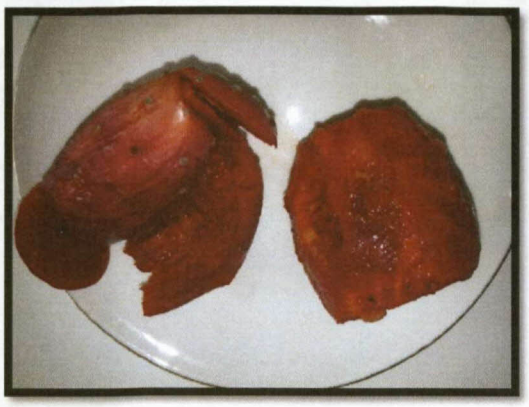
Green: *O. ficus-indica* Morado

Pink: *O. ficus-indica* Sicilian Indian Fig

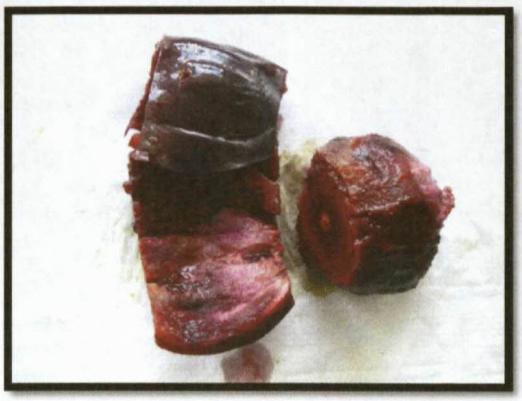
Pink: *O. ficus-indica* Meyers

Orange: *O. ficus-indica* Gymno-Carpo

Orange: *O. ficus-indica* Ofer



Purple: *O. ficus-indica* Nudosa



Purple: *O. robusta* Robusta

Figure 3.1: The eight cultivars (two fruit each selected from four fruit colours) included in the study of the fresh fruit (pulp, peel and seeds) and cladodes.

3.2.2 Sample Preparation

Three fruit per cultivar were thawed at 4 °C. The fruit and peel were liquidized with 50% and 100% distilled water, respectively, using a Milex 4-in-1 multi-purpose Mean Juice Machine (model MMJ004). The homogenized samples were strained (0.5 mm mesh size), the volume of the filtrate determined and aliquots were frozen at -18 °C until further analysis (not longer than one month) (De Wit *et al.*, 2010).

The separated seeds were washed, dried and ground using a Krups F03 Grinder. One gram of the powdered seeds was separated and used for carotene determinations. The remaining powder was weighed and five times the same weight of distilled water was added. It was vortexed for 60 s and homogenized for 30 s using a Janke & Kunkel Ultra-Turrax T25. It was then centrifuged at 4 °C using a 12 Hettich centrifuge for 10 min at 8000 rpm. The supernatant was frozen in aliquots in the same way as the peel and fruit.

Cladodes were weighed, cut into pieces and liquidized using the rotary blade from a Milex 4-in-1 multi-purpose Mean Juice Machine (model MMJ004) and further liquidized using a Salton Elite mixing wand. Equal amounts of water (100%) were added, homogenized with the Ultra-Turrax T25 for 30 s and centrifuged using a 12 Hettich centrifuge at 8000 rpm for 10 min at 4 °C. The supernatant was frozen in aliquots as described above.

3.3 Determination of Antioxidant content

3.3.1 Betalains

Betalain determination was done according to Castellanos-Santiago and Yahia (2008) and Stintzing *et al.* (2005). The aqueous extract (as discussed in 3.2.2) was centrifuged at 10 000 rpm for 5 min in a 12 Hettich centrifuge. The photometric quantification of the supernatant was done on a Genesys 10 Vis Thermo Spectronic spectrophotometer at an absorbance of 600 nm according to Stintzing *et al.* (2005). Measurements were done in triplicate and the betalain content (which comprises of the red-violet betacyanins and the yellow betaxanthins) was calculated according to the following equation and thereafter all values were converted to mg/kg (fresh weight).

$$Bc\left(\frac{mg}{g}\right) = A(DF)(MW)(1000)/E(L)$$

For calculating betacyanins:

$A\ is\ absoption\ value\ at\ 600nm$

$DF = Dilution\ factor$

$Molecular\ weight\ (MW) = 550$

$Molar\ extinction\ coefficients\ of\ betanin\ (E) = 60\ 000$

$L = pathlengh\ of\ the\ cuvette\ (1cm)$

For calculating betaxanthins:

$A\ is\ absoption\ value\ at\ 600nm$

$DF = Dilution\ factor$

$Molecular\ weight\ (MW) = 308$

$Molar\ extinction\ coefficients\ of\ indicaxanthin\ (E) = 48\ 000$

$L = pathlength\ of\ the\ cuvette\ (1cm)$

3.3.2 Ascorbic acid

Ascorbic acid was determined according to the 2,6 dichlorophenol indophenol titration method (James, 1995). A 10 fold and 100 -fold dilution of the aqueous extract (as explained in 3.2.2) was titrated with 0.04% 2,6 dichlorophenol-indophenol solution to a pink colour (titration volume T). The titration was repeated with 5 ml water as blank (Titration volume B) and with 5 ml ascorbic acid standard 0.02% solution (Titration volume St). Ascorbic acid was expressed as mg/100 g fresh weight using the following equation:

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

$T = titration\ value$

$B = blanc\ (0.02)$

$St = standard\ solution\ (5.18)$

$DF = Dilution\ factor$

3.3.3 Total phenolics

Total phenolics were determined using two grams of the aqueous extract (as discussed in 3.2.2). After the samples had been centrifuged in order to obtain a clear extract, 0.2 ml was combined with 1 ml Folin-Ciocalteu reagent and 0.8 ml sodium carbonate solution concentration. An absorbance reading at 765 nm was done in a Genesys 10 Vis Thermo Spectronic spectrophotometer after 30 min. The polyphenol reading at 765 nm was expressed as mg of gallic acid equivalents per liter (mg/l GAE), following a calibration curve with pure gallic acid at 0, 50, 100, 150, 200, 250, 300, 350 mg/l concentrations (Stintzing *et al.,* 2005). All values were converted to mg/kg (fresh weight).

3.3.4 Carotenoids

Carotenoids were analysed using the method described by Kuti (2004) and Fernández-López (2010). Two grams of cactus pear tissue from the fruit pulp, skin and cladodes and one gram of the seeds were homogenized using a Janke & Kunkel Ultra-Turrax T25 with 10 ml hexane/acetone/ethanol (50:25:25, v/v) before being centrifuged at 6500 rpm at 4°C for 5 min. The top layer of hexane, containing the colour, was recovered and transferred to a 25 ml volumetric flask. The volume of recovered hexane was then adjusted to 25 ml with hexane. Total carotenoid determination was carried out on the aliquot of hexane extract by measuring

absorbance at 450 nm using a Genesis 10 Vis Thermo Spectronic spectrophotometer. Total carotenoids were calculated using an extinction coefficient of β-carotene, $E^{1\%}$=2590 (Rodriquez-Amaya, 1999). The results were converted to µg/g (fresh weight).

$$x(\mu g) = A \cdot y(mL) \cdot 10^6 / A_{1cm}^{1\%} \cdot 100$$

$$x\left(\frac{\mu g}{g}\right) = \frac{x(\mu g)}{weight\ of\ sample}$$

$x = weight\ or\ concentration\ of\ carotenoid$

$A = absorbance$

$y = volume\ of\ solution\ (25\ ml)$

$A_{1cm}^{1\%} = 2590\ (absorption\ coefficient)$

3.3.5 Determination of antioxidant potential

3.3.5.1 Radical scavenging assay

Radical scavengers directly react with and scavenge peroxide radicals to terminate them. This test is a standard assay in antioxidant activity studies and offers a rapid, simple, sensitive and reproducible technique. The freshly prepared DPPH (2,2-dipehyl-1-picrylhydrazyl radical) is stable and has a deep purple colour with maximum absorption at 517 nm. The available antioxidants in the sample cause the purple colour to disappear by reducing it to a pale yellow colour. Thus, the antioxidants provide hydrogen atoms or donate electrons, comparable to a free-radical attack on the DPPH molecules and quench them to a colourless or bleached end product. The difference in colour is detected at 517 nm (Gülçin, 2012).

According to the methods of Sumaya-Martinez *et al.* (2011) and Morales and Jiménez-Pérez (2001) an ethanolic solution of DPPH (7.4 mg/100ml) was prepared. In each test, 500 µl of the DPPH solution was added to 100 µl of the aqueous samples (explained in 3.2.2). It was vortexed for 10 s and left to stand for one hour. It was then centrifuged using an EBA 12 Hettich centrifuge at 10 000 rpm for 5 min at 4°C before the absorbance was read at 517 nm using a Genesis 10 Vis Thermo Spectronic spectrophotometer (Gülçin *et al.*, 2007).

The method was sufficient for the seed and cladode determinations where the samples were colourless. In the fruit (pulp) and peel samples (fresh and processed) it was necessary to establish a blank reading that determined the level at which the colour of the sample measured

on the spectrophotometer, without the colour of the DPPH reagent, since both the red/purple colour of the fruit and the red/purple colour of the DPPH reagent appear on the spectrum at approximately 520 nm. The colour of the sample had to be taken into account when calculating the % DPPH level, since the colour and antioxidant have the same formation mechanisms; it may or may not be bleached, depending on whether the colour act as antioxidant during the DPPH experiment. In all fruit and peel samples, two samples for each reading were prepared simultaneously, one containing the sample with all the reagents as indicated above, and one with the sample, and the dilutant for the reagent (ethanol), but no reagent. The resultant blank samples were treated in exactly the same way, and were the same volume as the active sample. The reading of the blank sample was deducted from the reading of the reactive sample in order to calculate the true amount of DPPH bleaching. Dilution factors in pink and purple cultivar samples between 20 and 220 were necessary in order to achieve a spectrophotometer reading of under 0.2. The capacity to scavenge the DPPH radical was determined in this study using the following equation in order to rule out the contribution of the different colour samples:

$$DPPH\ scavenge\ effect\ (\%) = \left(\frac{Acontrol - (Asample - Ablanc)}{Acontrol} x\ 100\right) x\ DF$$

$Acontrol =$ *absorbance of the reagent (approximately* 1.8)

$Asample =$ *absorbance of the sample, dilutants and reagent*

$Ablank =$ *absorbance of sample with dilutants without reagent*

3.3.5.2 Chelating activity of ferrous ions

Excessive ferrous irons (Fe^{2+}) in plant, animal and human systems can cause the production of free radicals. An effective ion chelator is not an antioxidant but could prevent the oxidative damage by removing the free iron ions. Ferritin is the single most iron-storing protein that functions by forming complexes with Fe^{2+}. Chelating agents stabilize the oxidized form of the metal ions therefore they are effective as secondary antioxidants (Gülçin, 2012).

The method described by Gülçin *et al.* (2007) was used in the present study in accordance with the study of Sumaya-Martinez *et al.* (2011) in order to compare results in different colour fruit from Mexico and South Africa, respectively. One hundred µl of the samples (as previously described in 3.2.2) were prepared and 50 µl ferric (II) chloride solution (2 mM) and 4.5 ml methanol was added. It was vortexed for 10 s and 200 µl ferrozine (5 mM) was added. It was centrifuged using an EBA 12 Hettich centrifuge at 10 000 rpm for 5 min at 4˚C and the absorbance was read at 562 nm in a Genesis 10 Vis Thermo Spectronic spectrophotometer.

The method described by Gülçin *et al.* (2007) was acceptable for samples without red/purple pigmentation, but for Meyers (pink), Nudosa (purple) and Robusta (purple) samples is was necessary to dilute the samples and to obtain a blank reading for each sample. Two samples for each reading were prepared simultaneously, one containing the sample with all the reagents as indicated above, and one with the sample, and the dilutants for reagents (methanol), but no reagents. The resultant blank samples were treated in exactly the same way than the reactive samples, and were the same volume. Therefore 100 µl sample was added to 50 µl methanol and 4.5 ml methanol and after mixing, 200 µl methanol was added.

All tests were run in triplicate. The percentage of inhibition of the ferrozine-Fe^{2+} complex formation was calculated (Gülçin *et al.*, 2007). In the case where the samples were diluted, the dilution factor was multiplied with the answer obtained from the following formula:

$$Ferrous\ ions\ chelating\ effect\ (\%) = \left(\frac{Acontrol - (Asample - Ablank)}{Acontol} x\ 100\right) x\ DF$$

$Acontol = Absorbance\ of\ distilled\ water\ used\ instead\ of\ sample$

$(with\ ferric\ (II) chloride\ and\ ferrozine)$ (0.04)

$Asample =\ Absorbance\ in\ the\ presence\ of\ the\ sample\ and\ reagents$

$Ablank = Absorbance\ of\ sample\ with\ dilutants\ without\ reagents$

3.3.6 Statistical analysis

Data obtained for the amount of each antioxidant found in the different cactus pear cultivars were entered into an Excel spreadsheet. Data was analyzed using NCSS (2007) statistical software. Analysis of variance (ANOVA) was used to determine the significant differences between cultivars. Pearson correlation analysis tests were done in order to correlate each of the antioxidants with the antioxidant potential. In order to correlate the cultivars with the antioxidants that it contains, PCA (Principal Component Analysis) tests were done.

3.4 Results and discussion

The antioxidant content (Betalains, Ascorbic acid, Phenolics and Carotene) and capacity (% DPPH and % Chelating activity) will be determined and discussed in fresh products. To explain the effect of cultivar on the antioxidant contents and potential on fresh fruit, peel, seeds and cladodes the mean values of the individual potential tests and antioxidant contents are indicated

in Tables 3.1 to 3.4. The combined ANOVA (Table 3.5) will follow, offering an analysis of variance for the influence of fruit colour, cultivar and tissue type and the interaction between cultivar x tissue type on antioxidant properties of fresh cactus pear. Principal Component Analysis (Figure 3.3 to 3.6) will follow which will enable the cultivars to be correlated with the antioxidants that prevail in the fresh fruit, peel, seed and cladodes. The effect of colour on the antioxidant properties of fresh fruit will be thoroughly discussed using Table 3.6 and Figure 3.7, displaying mean values as well as a PCA. The Pearson correlation analysis (Table 3.7) will ascertain a measure of correlation strength between the individual antioxidant contents and -capacities. Finally the interaction between cultivar and tissue type on each individual antioxidant property will be discussed in order to offer rounded and complete information on fresh cactus pears.

3.4.1 The influence of cultivar on antioxidants of the various tissue types

It proved to be problematic to attain the required data for the antioxidant capacities (% DPPH and % Chelating activity) of different coloured fruit products, in this study. Since the reading of absorbance (using a Spectronic spectrophotometer) involves colour, the different colours of the pulp and peel affected the values in the case where the sample colour is not destroyed or bleached. The interference was most problematic in the orange, pink and purple fresh and processed samples that contain Betalains (more concentrated). Betacyanins (red/purple) peak on the visible light spectrum between 400 and 600 nm (540 nm), while Betaxanthins (yellow/orange) peak between 400 and 500 nm (480 nm) (Azeredo, 2009) (Figure 3.2). These absorption maxima ranges are where the absorbance of DPPH (517 nm) and ferrozine (562 nm) are determined. It was therefore important to ascertain the level at which the sample colour contributed to the spectrophotometric readings, before the influence of the reagent could be obtained. The method used in this study was chosen based on its use in other studies on similar cactus pear colours and cultivars: DPPH (Piga *et al.,* 2003, Yahia & Mondragon-Jacobo, 2011, Fernández-López *et al.,* 2010, Galati *et al.,* 2003) and % Chelating activity (Sumaya-Martinez *et al.,* 2011) yet the authors failed to mention this problem or to indicate the necessity of using blanks (even when blanks were used). It may thus appear as if it was not taken into account in their determinations and calculations. Therefore results from the current study, where blanks were used to determine the influence of colour, differ from that of other authors. In retrospect, ABST would have been a more suited method to use, since the spectrophotometer readings fall outside the spectrum of fruit colours (Joubert, 2012).

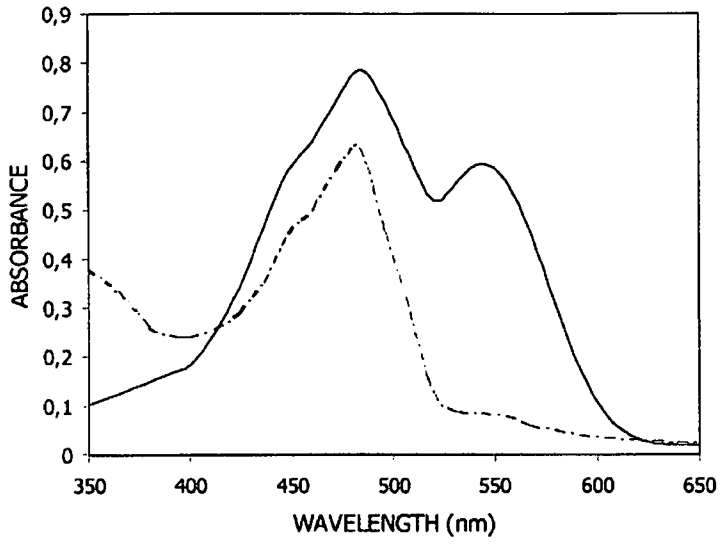


Figure 3.2: The visible light absoption spectra of betacyanins (solid line) and betaxanthin (dotted line) colours found in cactus pear fruit (Fernández-López & Almela, 2001)

3.4 1.1 The effect of cultivar on the antioxidant properties of fresh cactus pear fruit

To explain the effect of cultivar on antioxidant content and -potential on fruit (pulp), the mean values of the individual potential tests and antioxidant contents for the different cultivars are indicated in Table 3.1.

Regarding the antioxidant potential, the percentage (%) DPPH antioxidant activity for fresh fruit as seen in Table 3.1, were significantly different. Morado (green) significantly differed from all other cultivars as it had the least activity (57.05%) while Nepgen (green) and Nudosa (purple) measured the highest antioxidant activity amongst all other cultivars with values of respectively 83.63 and 82.47%. There were only some similarities to the findings by Sumaya-Martinez *et al.* (2011). In their study, all green and yellow fruit had very low DPPH levels, while in this study there were high values amongst green cultivars. All purple and red fruit differed significantly from each other with some cultivars having high and low values (reported by Sumaya-Martinez *et al.*, 2011); this trend was comparable to all cultivars in this study.

The percentage Chelating activity did not differ significantly amongst all the cultivars but was overall high with an average of 93.86%. The highest value measured was for Nudosa (purple) (96.68%) and the lowest for Gymno-Carpo (orange) (89.17%). These were higher than what was found by Sumaya-Martinez *et al.* (2011) where the lowest value was 51% and the highest

value was 74%, but as was found in this study, no significant differences were found amongst all cultivars tested.

As for the Phenolic levels, Sicilian Indian Fig (1.68 mg/kg GAE) and Nudosa (2.85 mg/kg GAE) had significantly lower values, while Gymno-Carpo (22.08 mg/kg GAE) had the highest levels. These values were lower than Phenolic values found in literature where the Phenolics in cactus pear fruit ranged from 21.88 mg/kg (Fernández-López *et al.*, 2010) to 746 mg/kg (Galati *et al.*, 2003). A number of researchers found the highest levels of Phenolics in red/purple fruit; Stintzing *et al.* (2005); Alimi *et al.* (2012); Yahia and Mondragon-Jacobo (2011); Chavey-Santoscoy *et al.* (2009); Morales and Sáenz (2009); Coria-Cayupán *et al.* (2011) and Kuti (2004), but that trend was not evident in the current study (Table 3.1).

For Betalains (Betacyanins and Betaxanthins) it is clear that Robusta had approximately 41 times higher levels than all other cultivars (126.6 mg/kg) and is an excellent source of betalains. Even though Robusta measured very high levels of betalains in this study, the same cultivar measured 1140.4 mg/kg for combined Betacyanins and Betaxanthins in a study by Stintzing *et al.* (2005). Though, it may be noted that two of the other pink cultivars (Meyers and Nudosa) had slightly (but not significantly) higher levels than other colour cultivars.

The two orange coloured cultivars, Ofer (94.07 mg/100 g) and Gymno-Carpo (95.27 mg/100 g), had significantly higher ascorbic acid levels than other coloured cultivars, but is was surprising that one green coloured cultivar (Nepgen) had statistically similar levels of ascorbic acid to the orange cultivars (91.69 mg/100 g). The other green cultivar included in the study (Morado) had the lowest levels of ascorbic acid (12.11 mg/100 g). Ascorbic acid levels found in this study was higher than the reported levels in related studies where measured Ascorbic acid ranged from 0.79 mg/100 g for red cactus fruit (Kuti, 2004) to 48 mg/100 g for purple fruit (Coria-Cayapán *et al.*, 2011). White fruit (in this study called green fruit), had a value of 2.56 mg/100 g in a study by Stintzing *et al.* (2005). In an earlier study done on cultivars from the same orchard as the current study in 2010, Ascorbic acid content ranged from 0.85 to 4.12 mg/100 g but similarities in trends were found with De Wit *et al.* (2010), namely that Gymno-Carpo demonstrated the highest values of Ascorbic acid.

Carotene levels were very low (between 0.62 and 2.38 µg/g) in all fresh cactus pear fruit and there were no significant differences between cultivars. Nudosa (purple) measured the highest levels at 2.38 ug/g. These findings were similar as was found by numerous researchers. Generally it was found that Carotene levels were low in cactus pear fruit with levels ranging from

0.00145 ug/g (Tesoriere *et al.,* 2005) to 17.7 ug/g (Kuti, 2004). In many studies, purple fruit were determined to have the highest levels of carotene (comparative to what was found in this data) (Yahia and Mondragon-Jacobo, (2011) Morales and Sáenz (2009), Figueroa-Cares *et al.* (2010), Stintzing and Carle (2005), Fernández-López *et al.* (2010), Alimi *et al.* (2012) and Kuti (2004).

Table 3.1: The effect of cultivar on the antioxidant properties of fresh cactus pear fruit.

Cultivar	DPPH (n=3) %	Chelating activity (n=3) %	Phenolics (n=3) mg/kg GAE	Betacyanins (n=3) mg/kg	Betaxanthins (n=3) mg/kg	Betacyanins + Betaxanthins (n=3) mg/kg	Ascorbic Acid (n=3) mg/100g	Carotene (n=3) ug/g
Nepgen (Green)	83.63 ± 1.30^{d}	94.94 ± 0.37	16.72 ± 7.48^{ab}	1.83 ± 0.55^{a}	1.28 ± 0.39^{a}	3.11 ± 0.94^{a}	91.69 ± 8.98^{b}	0.62 ± 0.19
Meyers (Pink)	58.65 ± 11.49^{ab}	93.33 ± 5.20	17.45 ± 9.15^{ab}	2.71 ± 0.15^{a}	1.90 ± 0.10^{a}	4.62 ± 0.25^{a}	52.52 ± 5.75^{ab}	0.92 ± 0.16
Ofer (Orange)	77.56 ± 4.61^{cd}	94.17 ± 3.82	13.41 ± 1.62^{ab}	1.20 ± 0.17^{a}	0.84 ± 0.12^{a}	2.05 ± 0.28^{a}	94.07 ± 29.97^{b}	1.69 ± 0.32
Gymno C (Orange)	70.12 ± 4.99^{abcd}	89.17 ± 7.64	22.08 ± 11.81^{b}	1.54 ± 0.28^{a}	1.08 ± 0.20^{a}	2.62 ± 0.48^{a}	95.27 ± 21.00^{b}	1.86 ± 0.09
Robusta (Purple)	77.05 ± 4.53^{bcd}	95.09 ± 0.38	18.47 ± 6.02^{ab}	74.47 ± 41.06^{b}	52.13 ± 28.74^{b}	126.60 ± 69.81^{b}	32.38 ± 22.99^{a}	1.58 ± 0.09
Morado (Green)	57.05 ± 11.19^{a}	95.00 ± 2.50	8.46 ± 2.66^{ab}	0.72 ± 0.14^{a}	0.51 ± 0.10^{a}	1.23 ± 0.25^{a}	12.11 ± 0.24^{a}	0.82 ± 0.48
Sic In Fig (Pink)	59.73 ± 2.04^{abc}	92.50 ± 5.00	1.68 ± 1.79^{a}	0.89 ± 0.27^{a}	0.62 ± 0.19^{a}	1.51 ± 0.46^{a}	15.47 ± 1.92^{a}	1.47 ± 0.25
Nudosa (Purple)	82.47 ± 4.00^{d}	96.68 ± 0.42	2.85 ± 3.53^{a}	3.76 ± 1.01^{a}	2.64 ± 0.71^{a}	6.40 ± 1.72^{a}	32.82 ± 18.37^{a}	2.38 ± 1.66
AVG	70.78	93.86	12.64	10.89	7.62	18.52	53.29	1.42
STD	11.85	4.02	9.02	27.39	19.17	46.56	36.89	0.78
CV	16.73	4.28	71.37	251.49	251.49	251.49	69.21	54.65
Significance level	$p < 0.001$	NS	$p < 0.05$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	NS

Means with different superscripts in the same column differ significantly NS = Not Significantly

3.4.1.2 The effect of cultivar on the antioxidant properties of fresh cactus pear peel

To explain the effect of cultivar on antioxidant content and -potential on peel, the mean values of the individual potential tests and antioxidant contents for the different cultivars are indicated in Table 3.2.

Percentage DPPH for the peel was not significantly different amongst all cultivars (Table 3.2) although it was consistently high (91 to 96%) and in comparison to the fruit (pulp), it showed higher antioxidant capacity levels. Meyers (pink) and Nudosa (purple) demonstated the highest levels at 96.25% and 96.85% respectively.

The % Chelating activity was lower than in the fruit (pulp); Ofer had the lowest (significantly different) levels (69.17%) while both the purple cultivars namely Robusta (97.32%) and Nudosa (96.63%) tested the highest capacity for chelating ability.

Meyers had statistically the highest Phenolic levels (58.88 mg/kg GAE). These were the highest Phenolic contents measured among all cultivars for both fruit and peel. The average Phenolic levels were higher in peel (21.58 mg/kg GAE) (Table 3.2) than in fruit (12.64 mg/kg GAE) (Table 3.1).

Robusta had very high levels of Betalains in the peel, although the difference between Robusta and the other cultivars was not as big as was seen in fruit (pulp) levels. Levels were only 14 times higher (in comparison to 41 times) than the mean levels of all the other cultivars as the peel of the other cultivars seemed to contain more Betalains especially in the pink cultivars. The Robusta peel contained considerably less Betalains than the Robusta fruit (pulp) (Tables 3.1 and 3.2).

As for Ascorbic acid, Meyers (pink) (86.28 mg/100 g) had the highest content in the peel (significantly different) while Morado (green) (29.23 mg/100 g) and Sicilian Indian Fig (pink) (24.22 mg/100 g) had the least.

Carotene levels were consistently higher in the peel (average 3.48 ug/g) than the fruit (pulp) (average 1.42 ug/g). Robusta showed the highest (6.06 ug/g) and Meyers the lowest levels in the peel of fresh fruit (1.79 ug/g) (Tables 3.1 and 3.2).

Table 3.2: The effect of cultivar on the antioxidant properties of fresh cactus pear peel.

Cultivar	DPPH (n=3) %	Chelating activity (n=3) %	Phenolics (n=3) mg/kg GAE	Betacyanins (n=3) mg/kg	Betaxanthins (n=3) mg/kg	Betacyanins + Betaxanthins (n=3) mg/kg	Ascorbic Acid (n=3) mg/100g	Carotene (n=3) ug/g
Nepgen (Green)	91.67 ± 1.04	81.67 ± 5.20^{abc}	15.96 ± 8.06^{a}	0.89 ± 0.37^{a}	0.62 ± 0.26^{a}	1.52 ± 0.63^{a}	55.88 ± 7.71^{ab}	3.46 ± 0.40^{ab}
Meyers (Pink)	96.25 ± 2.91	70.00 ± 10.90^{ab}	58.88 ± 25.60^{b}	6.87 ± 0.75^{a}	4.81 ± 0.53^{a}	11.69 ± 1.28^{a}	86.28 ± 21.90^{b}	1.79 ± 0.35^{a}
Ofer (Orange)	93.85 ± 0.75	69.17 ± 3.82^{a}	21.31 ± 14.73^{a}	1.11 ± 0.22^{a}	0.78 ± 0.15^{a}	1.89 ± 0.37^{a}	64.24 ± 16.73^{ab}	4.80 ± 0.59^{ab}
Gymno C (Orange)	91.18 ± 2.11	72.50 ± 9.01^{ab}	14.04 ± 2.48^{a}	2.21 ± 0.21^{a}	1.55 ± 0.15^{a}	3.75 ± 0.37^{a}	68.04 ± 38.66^{ab}	3.99 ± 1.32^{ab}
Robusta (Purple)	91.65 ± 0.44	97.32 ± 0.38^{c}	7.44 ± 4.48^{a}	42.62 ± 8.79^{b}	29.84 ± 6.15^{b}	72.46 ± 14.94^{b}	61.16 ± 25.48^{ab}	6.06 ± 2.83^{b}
Morado (Green)	91.70 ± 2.20	87.50 ± 9.01^{bc}	31.88 ± 18.22^{ab}	1.84 ± 0.73^{a}	1.29 ± 0.51^{a}	3.13 ± 1.23^{a}	29.23 ± 3.89^{a}	3.59 ± 0.92^{ab}
Sic In Fig (Pink)	90.95 ± 8.41	87.50 ± 2.50^{bc}	12.56 ± 2.04^{a}	3.23 ± 0.27^{a}	2.26 ± 0.19^{a}	5.49 ± 0.47^{a}	24.22 ± 5.89^{a}	2.11 ± 0.14^{a}
Nudosa (Purple)	96.85 ± 2.18	96.63 ± 0.13^{c}	10.59 ± 1.40^{a}	5.08 ± 1.55^{a}	3.55 ± 1.09^{a}	8.63 ± 2.64^{a}	46.40 ± 11.47^{ab}	2.01 ± 0.58^{a}
AVG	93.01	82.78	21.58	7.98	5.59	13.57	54.43	3.48
STD	3.65	12.10	19.30	13.78	9.64	23.42	25.67	1.74
CV	3.93	14.62	89.42	172.57	172.57	172.57	47.16	50.17
Significance level	NS	$p < 0.001$	$p < 0.01$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.05$	$p < 0.01$

Means with different superscripts in the same column differ significantly NS = Not Significantly

3.4.1.3 The effect of cultivar on the antioxidant properties of fresh cactus pear seeds

To explain the effect of cultivar on antioxidant content and -potential on seeds, the mean values of the individual potential tests and antioxidant contents for the different cultivars are indicated in Table 3.3.

In Table 3.3 it can be seen that the measured levels of all antioxidants in seeds are greatly elevated from that of fruit (pulp) (Table 3.1) and peel (Table 3.2), however it must be kept in mind that firstly, all seed samples were dried, therefore the measurements are calculated for dried weight whereas other tissue measurements were calculated as per fresh weight. Secondly, the seeds were ground, allowing the contents to be dissolved in the media. The question remains whether the antioxidants present in seeds are available to the body if the seeds remain intact as it travels through the gastro-intestinal tract. A third consideration is that there are at maximum 7 g of seeds found in whole cactus fruit (Nobel, 2002), therefore levels measured per 100 g, that are applicable to a single fruit and only a tenth of a cladode, would indicate the seeds of at least 14 fruit. It is therefore important to look at trends rather than actual measured amounts.

Neither the DPPH, nor the Chelating activity percentages differed significantly. The percentage DPPH average (40.48%) (Table 3.3) is much lower compared to fruit (70.78%) (Table 3.1) and peel (93.01%) (Table 3.2), with the Chelating activity being slightly lower in seeds. These findings reiterate the fact that seeds do not have a protective function in plants but rather a nutrient providing function.

Nevertheless, the Phenolic and Carotene levels are highly elevated in seeds, indicating that products made from seeds such as oil may be good sources of Phenolics and Carotene. The highest Phenolic levels were found in Morado (291.46 mg/kg) and the lowest in Nudosa (74.85 mg/kg) but none of the readings were statistically significant.

Table 3.3: The effect of cultivar on the antioxidant properties of fresh cactus pear seed.

Cultivar	DPPH (n=3) %	Chelating activity (n=3) %	Phenolics (n=3) mg/kg GAE	Betacyanins (n=3) mg/kg	Betaxanthins (n=3) mg/kg	Betacyanins + Betaxanthins (n=3) mg/kg	Ascorbic Acid (n=3) mg/100g	Carotene (n=3) ug/g
Nepgen (Green)	42.35 ± 9.13	94.17 ± 1.44	184.30 ± 31.76	69.89 ± 33.60^{a}	48.92 ± 23.52^{a}	118.81 ± 57.13^{a}	28.98 ± 0.36^{a}	40.15 ± 2.84^{a}
Meyers (Pink)	40.20 ± 12.99	90.00 ± 5.00	276.63 ± 76.42	39.01 ± 14.50^{a}	27.31 ± 10.15^{a}	66.32 ± 24.66^{a}	83.22 ± 22.72^{bc}	66.54 ± 13.08^{bc}
Ofer (Orange	39.25 ± 10.14	88.33 ± 3.82	258.83 ± 30.32	35.58 ± 17.16^{a}	24.91 ± 12.01^{a}	60.49 ± 29.17^{a}	86.75 ± 36.14^{c}	32.59 ± 9.84^{a}
Gymno C (Orange)	48.64 ± 12.36	90.83 ± 5.20	278.73 ± 55.34	22.41 ± 3.75^{a}	15.69 ± 2.63^{a}	38.10 ± 6.38^{a}	49.91 ± 17.54^{abc}	43.31 ± 13.74^{ab}
Robusta (Purple)	56.50 ± 17.85	85.83 ± 6.29	258.35 ± 168.32	160.88 ± 69.85^{b}	112.62 ± 48.89^{b}	273.50 ± 118.74^{b}	36.12 ± 10.64^{ab}	41.67 ± 6.17^{ab}
Morado (Green)	32.39 ± 6.24	91.67 ± 1.44	291.46 ± 70.86	17.48 ± 6.55^{a}	12.24 ± 4.59^{a}	29.72 ± 11.14^{a}	29.72 ± 1.12^{a}	47.04 ± 2.12^{abc}
Sic In Fig (Pink)	26.68 ± 11.01	83.33 ± 18.09	204.47 ± 88.92	56.50 ± 19.02^{a}	39.55 ± 13.32^{a}	96.04 ± 32.34^{a}	39.68 ± 15.87^{abc}	69.24 ± 3.72^{c}
Nudosa (Purple)	37.86 ± 4.66	84.17 ± 3.82	74.85 ± 75.97	23.86 ± 8.33^{a}	16.70 ± 5.83^{a}	40.56 ± 14.16^{a}	28.22 ± 1.30^{a}	45.62 ± 12.32^{abc}
AVG	40.48	88.54	228.45	53.20	37.24	90.44	47.83	48.27
STD	12.84	7.26	99.13	51.26	35.88	87.15	27.29	14.49
CV	31.71	8.20	43.39	96.36	96.36	96.36	57.05	30.03
Significance level	NS	NS	NS	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.01$	$p < 0.01$

Means with different superscripts in the same column differ significantly NS = Not significant

It is interesting to observe that although Robusta seeds do not seem to be purple in colour, the betalains found in this cultivar were significantly higher than the other cultivars ($p < 0.001$) clearly indicating the effect of cultivar on seed antioxidant content.

Nepgen (28.98 mg/100 g), Morado (29.72 mg/100 g) and Nudosa (28.22 mg/100 g) had statistically the lowest Ascorbic acid values and Ofer the highest (86.75 mg/100 g) (significance level at $p < 0.01$). The Ascorbic acid content was on average lower than fruit (Table 3.1) and peel (Table 3.2) but with an average of 47.83 mg/100 g it would be considered a good source of Ascorbic acid nevertheless.

On average the Carotene content in the seeds (48.27 ug/g) were much higher than the content in fruit (1.42 µg/g) and peel (3.48 µg/g) (Tables 3.1, 3.2 and 3.3), but it should be considered that the seeds were dried and ground prior to carotene determinations, therefore the carotene content would be higher than fruit and peel that were determined on fresh weight. Nepgen (40.15 µg/g) and Ofer (32.59 µg/g) had the lowest Carotene levels while Sicilian Indian Fig (69.24 µg/g) had the highest (statistically significant) values.

3.4.1.4 The effect of cultivar on the antioxidant properties of fresh cactus pear cladodes

To explain the effect of cultivar on antioxidant content and potential on cladodes, the mean values of the individual potential tests and -antioxidant contents for the different cultivars are indicated in Table 3.4.

The antioxidant activity in the cladodes measured by percentage DPPH was as high as in the fruit (pulp) and peel (Tables 3.1 and 3.2) as shown in Table 3.4. Morado (green) had the highest (significantly different) levels (95.31%) and this could be explained by the high Phenolic and relatively high Betalain, Ascorbic acid and Carotene levels found for Morado in the cladodes. Ofer had the lowest levels (not significantly different) at 83.77%. When Santos-Zea *et al.* (2011) determined the oxygen radical absorbance capacity using ORAC, O. *robusta* Galia had the highest (statistically significant) value (738.83 µmol/g TE) and *Opuntia ficus-indica* Nueva the lowest (264.56 µmol/g TE). In the study by Santos-Zea *et al.* (2011), the higher phenols levels were correlated with higher antioxidant capacity; that trend is also seen in this study as Morado had the highest Phenolics as well as % DPPH levels.

The chelating activity for Morado was significantly lower (40.77%) but Sicilian Indian Fig (27.44%) had the lowest (statistically significant) ability to chelate ferrous ions. Nepgen (89.17%) cladodes would contribute the most toward this antioxidant capacity but all the

cultivars had significantly different levels that ranged from 27.44 to 89.17%. No literature was available on the chelating ability in cladodes.

The Phenolic levels are noteworthy as they are extremely high, in some cases three times that of fruit (Table 3.1). Robusta (42.84 mg/kg) cladodes had the lowest while Morado (324.87 mg/kg) and Sicilian Indian Fig (304.41 mg/kg) had significantly high levels. Santos-Zea *et al.* (2011) determined the phenolic content in the cladodes of nine different varieties of Opuntia spp. and found the lowest values as 318 µg/g GAE and the highest at 905.08 µg/g GAE. *Opuntia robusta* H.L. Wendl var. Gavia had a Phenolic content value of 561.89 µg/g GAE and *Opuntia robusta* H.L. Wendl var. Tapón measured 689.46 µg/g GAE. It was stressed by Santos-Zea *et al.* (2011) that other researchers found 2-15 times higher values than in their study. As compared to this study though, the values are similar.

Betalain levels seem to even out in cladodes as the Robusta cladodes (29.20 mg/kg) has approximately the same and statistically similar contents as other cultivars (average 16.17 mg/kg). Nepgen had the lowest content (8.07 mg/kg). No literature was available on Betalain values in cladodes.

Ascorbic acid levels are lower than in fruit and peel (Tables 3.1 and 3.2), but averaged the same as seeds (respective averages were 43.56 and 47.83 mg/100g) (Table 3.3). The highest levels (significantly different) were found in Gymno-Carpo (77.40 mg/100g) and Meyers (67.25 mg/100g) cladodes. The lowest levels in cladodes were measured in Nudosa cladodes (19.15 mg/100g). Stintzing and Carle (2005) reported between 7 and 22 mg/100g in fresh cladodes and Medina-Torres *et al.* (2011) reported 2.05 g/kg (205 mg/100g).

Carotene content was considerably higher in cladodes than fruit and peel (Tables 3.1 and 3.2) with Meyers (18.15 µg/g), Ofer (17.87 µg/g) and Gymno Carpo (17.87 µg/g) measurements almost reaching 20 µg/g. The measurements were lower than what was found in seeds, with seeds average at 48.27 µg/g and cladodes 12.69 µg/g (Table 3.3). The lowest Carotene content levels were for Nepgen (6.72 µg/g). Bensadón *et al.* (2010) found Carotene content in young cladodes at 21.32 mg/g (21 320 µg/g). In agreement with this study, no significant differences were found in cladodes amongst different varieties. Jaramillo-Flores *et al.* (2003) reported carotene values in cladodes of 231 ug/g and Medina-Torres *et al.* (2011) of 1.16 g/kg (1160 ug/g).

Table 3.4: The effect of cultivar on the antioxidant properties of fresh cactus pear cladode.

Cultivar	DPPH (n=3) %	Chelating activity (n=3) %	Phenolics (n=3) mg/kg GAE	Betacyanins (n=3) mg/kg	Betaxanthins (n=3) mg/kg	Betacyanins + Betaxanthins (n=3) mg/kg	Ascorbic Acid (n=3) mg/100g	Carotene (n=3) ug/g
Nepgen (Green)	94.49 ± 1.37^{ab}	89.17 ± 1.44^{d}	241.53 ± 32.75^{bc}	4.74 ± 0.39^{a}	3.32 ± 0.27^{a}	8.07 ± 0.66^{a}	26.78 ± 1.01^{ab}	6.72 ± 1.50^{a}
Meyers (Pink)	92.74 ± 3.27^{ab}	80.83 ± 2.89^{cd}	257.25 ± 60.00^{bc}	7.54 ± 1.97^{ab}	5.28 ± 1.38^{ab}	12.82 ± 3.34^{ab}	67.25 ± 18.65^{c}	18.15 ± 7.20^{b}
Ofer (Orange)	83.77 ± 10.41^{a}	80.83 ± 7.22^{cd}	239.47 ± 17.12^{bc}	3.17 ± 2.24^{a}	16.98 ± 1.26^{c}	20.15 ± 2.78^{ab}	58.74 ± 11.14^{bc}	17.87 ± 1.30^{b}
Gymno C (Orange)	95.53 ± 0.15^{b}	77.50 ± 2.50^{cd}	270.93 ± 117.99^{bc}	3.17 ± 2.24^{a}	2.22 ± 1.56^{a}	5.40 ± 3.80^{a}	77.40 ± 11.34^{c}	17.87 ± 1.30^{b}
Robusta (Purple)	92.50 ± 0.91^{ab}	70.00 ± 9.01^{c}	42.84 ± 18.92^{a}	17.18 ± 7.74^{b}	12.02 ± 5.42^{bc}	29.20 ± 13.16^{b}	24.04 ± 7.44^{a}	11.29 ± 3.04^{ab}
Morado (Green)	95.31 ± 0.15^{b}	40.77 ± 1.33^{b}	324.87 ± 20.94^{c}	14.82 ± 4.84^{b}	10.37 ± 3.39^{bc}	25.20 ± 8.23^{b}	47.78 ± 11.52^{abc}	11.58 ± 0.85^{ab}
Sic In Fig (Pink)	94.08 ± 0.93^{ab}	27.44 ± 4.44^{a}	304.41 ± 96.43^{c}	9.19 ± 1.91^{ab}	6.44 ± 1.34^{ab}	15.63 ± 3.24^{ab}	27.31 ± 10.21^{ab}	7.29 ± 0.51^{a}
Nudosa (Purple)	93.91 ± 0.38^{ab}	80.26 ± 1.94^{cd}	131.32 ± 23.48^{ab}	7.59 ± 0.70^{ab}	5.31 ± 0.49^{ab}	12.90 ± 1.19^{ab}	19.15 ± 12.43^{a}	10.70 ± 1.08^{ab}
AVG	92.79	68.35	226.58	8.43	7.74	16.17	43.56	12.69
STD	4.89	21.44	103.13	5.78	5.19	9.29	23.34	5.12
CV	5.27	31.37	45.52	68.56	67.08	57.47	53.58	40.40
Significance level	$p < 0.05$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.01$	$p < 0.001$	$p < 0.001$

Means with different superscripts in the same column differ significantly

3.4.2 Combined ANOVA for colour, cultivar and tissue type

Analysis of variance was done on the data for the various colours, cultivars and tissue types as well as on their interactions (Table 3.5). Colour had a significant ($p < 0.01$ and $p < 0.05$) effect on the % DPPH and the Chelating activity demonstrated by the cactus pear plant while highly significant differences were found for all the antioxidant contents tested. The antioxidant content differs according to the colour of the fruit but the antioxidant capacity was less dependant on colour.

Cultivar had a highly statistically significant effect ($p < 0.001$) on the antioxidant capacity as well as the –content, consistently with every test performed. These results indicated that the antioxidants that are predominant in a specific cultivar are highly dependent on the cultivar.

The tissue type that was investigated namely the pulp, peel, seed and cladode had a highly statistically significant ($p < 0.001$) effect on the antioxidant type and -capacity and most of the individual antioxidants, except for Ascorbic acid. This indicated that Ascorbic acid could have high or low readings in any part of the plant and the probability is high that correlations that do exist happened by chance.

The cultivar x tissue type relationship was investigated and it was found that strong statistically significant differences ($p < 0.001$) are found with all of the antioxidant contents and antioxidant capacity tests. The colour x tissue type relationship analysis revealed that the Chelating ability and the Phenolic content were statistically significant ($p < 0.01$) and only Carotene content was statistically highly significantly different ($p < 0.001$). The remaining antioxidants (Betalains and Ascorbic acid) and antioxidant capacity (% DPPH) were not statistically significantly different and the chance that a relationship between the colour, the tissue type and these antioxidants exist, is weak.

Table 3.5: **Analysis of variance (ANOVA) for the influence of fruit colour, cultivar, tissue type and the interaction between cultivar and tissue type on antioxidant properties of fresh cactus pear.**

Antioxidant property:	Colour:	Cultivar:	Tissue Type:	Cultivar X Tissue type:	Colour X Tissue Type
% DPPH	**	***	***	***	NS
% Chelating activity	*	***	***	***	**
Phenolics	***	***	***	***	**
Betacyanins	***	***	***	***	NS
Betaxanthins	***	***	***	***	NS
Betacyanins + Betaxanthins	***	***	***	***	NS
Ascorbic acid	***	***	NS	***	NS
Carotene	***	***	***	***	***

NS = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

3.4.3 Principal component analysis (PCA)

Principal component analysis (PCA) condenses a large number of inconsistencies into a small number of main factors (Crossa, 1990). The variation is therefore explained in terms of factors that are common to all variables as well as in terms of unique factors, whereby principal components of the cultivar in terms of antioxidants are defined. The interaction is explained by plotting the principal component scores closely to each other (Crossa, 1990). The biplot makes it possible for cultivars to be correlated with the antioxidants that it contains. The aim of the PCA is to identify cultivars that are most associated with a specific antioxidant.

3.4.3.1 PCA of cultivar on the antioxidant properties of fresh cactus pear fruit

Factor 1 and 2 explained 67.44% of the variation (Figure 3.3). From the biplot, it is clear that the cultivars that are grouped in clusters around certain antioxidants are linked to those antioxidants. In fresh fruit (pulp), Gymno-Carpo is associated with Ascorbic acid and Phenolics, while Nepgen, Ofer and Meyers more closely associate with % DPPH and Carotene, but on the whole they all form a grouping. Meyers is only related to Carotene content (not closely). Nudosa, Morado and Sicilian Indian Fig are grouped together with % Chelating activity. Robusta is the only cultivar associated with Betalains.

In conclusion, the main associations in cactus pear fruit were: Gymno-Carpo, Nepgen and Ofer associated with Ascorbic acid, while Nepgen and Ofer also associated with % DPPH. Morado, Nudosa and Sicilian Indian Fig were closely associated with the highest % Chelating ability and Robusta with Betalains. These associations can be correlated with Table 3.1 in terms of the highest values for each antioxidant.

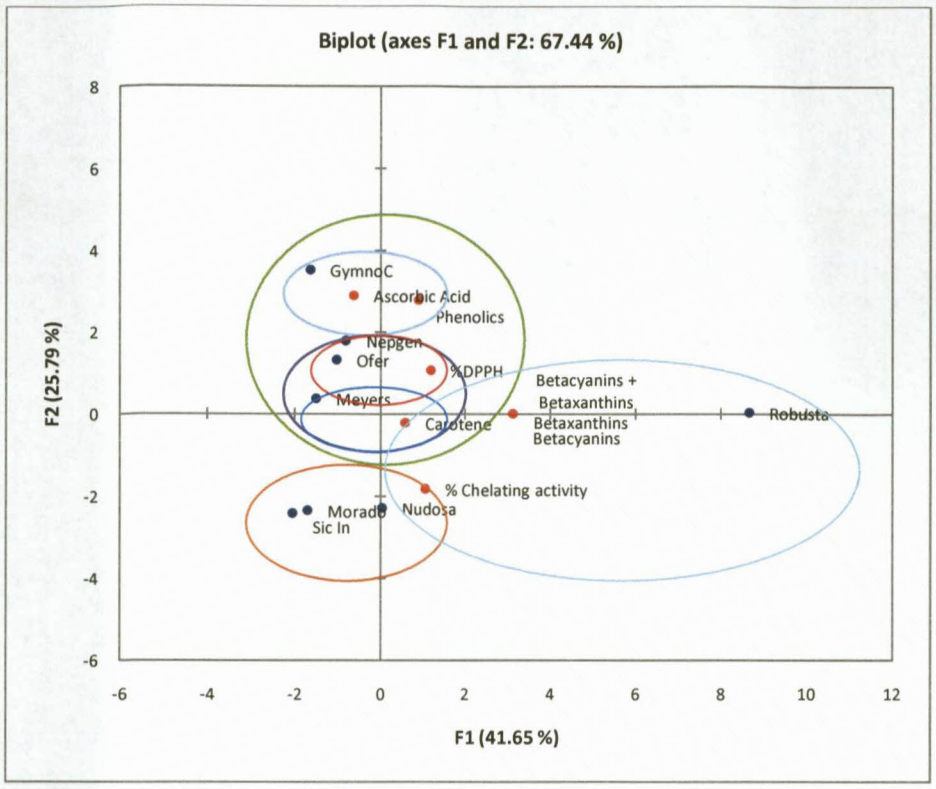


Figure 3.3: Principal component analysis of cultivar on the antioxidant properties of fresh cactus pear fruit.

3.4.3.2 PCA of cultivar on the antioxidants properties of fresh cactus pear peel

In the PCA for the peel (Figure 3.4) the variation is explained (75.24%) by Factors 1 and 2.

While Robusta remains associated with Betalains, it also grouped with Carotene and % Chelating activity. Meyers, Ofer, Gymno-Carpo, Nudosa, Nepgen, Morado and Sicilian Indian Fig are grouped together with Phenolics, Ascorbic acid and % DPPH. Ofer, Gymno-Carpo, Nudosa, Nepgen, Morado and Sicilian Indian Fig also seem to be linked with Carotene and % Chelating activity. Meyers associate with Ascobic acid, Phenolics and % DPPH, but not with Carotene and % Chelating activity as most of the other cultivars do.

It is clear that, in the peel, the variables are more clustered together than in the PCA for fruit (Figure 3.3). The associations and groupings found between antioxidant content and capacity become very clear in Figure 3.4: Ascorbic acid, Phenolics and % DPPH group together, while Carotene, Betalain and % Chelating activity associate.

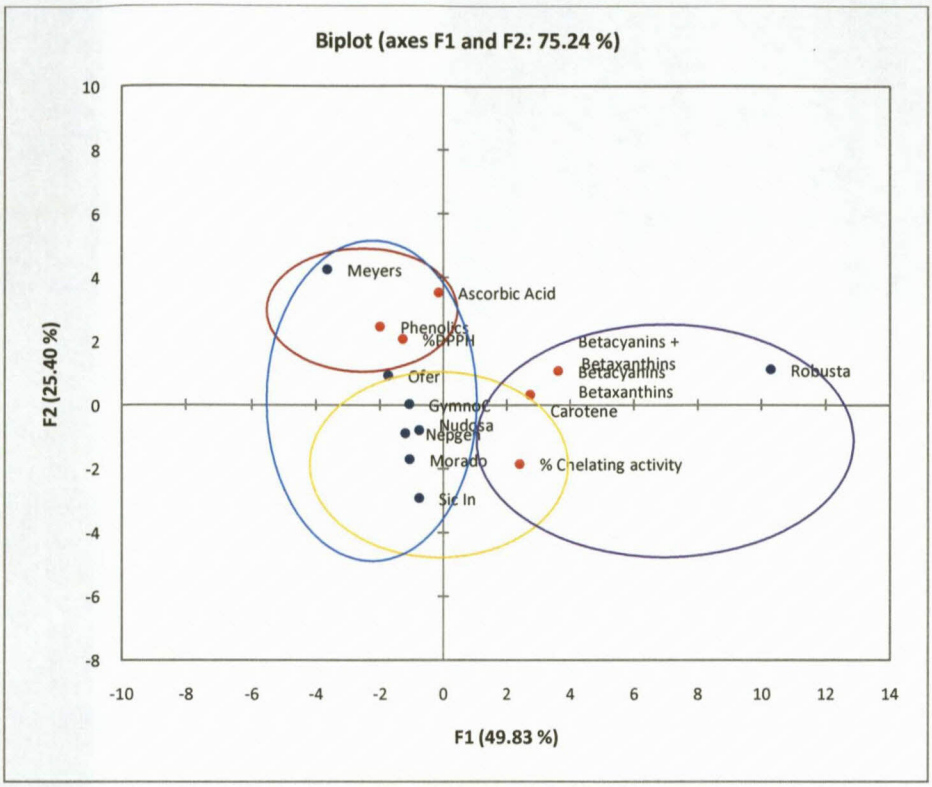


Figure 3.4: Principal component analysis of cultivar on the antioxidant properties of fresh cactus pear peel.

3.4.3.3 PCA of cultivar on the antioxidant properties of fresh cactus pear seed

In the biplot for seeds, factor 1 and 2 explained 67.84% of the variation (Figure 3.5).

The antioxidants and antioxidant capacity variables were bundled together in the biplot for seeds. This indicated that Phenolics, Ascorbic acid and % DPPH that associated in fruit (Figure 3.3) and peel (Figure 3.4) also associated in the seeds, but that % Chelating activity, Carotene and Betalains also associated closely with this group. Therefore it may be stated that, in seeds, most cultivars would contain high amounts of all the antioxidants that were included in this study as well as have good antioxidant capacity. There were three cultivars that could not be included in this statement: Robusta (purple) that seems to only associate with Betalains and Nudosa (purple) together with Sicilian Indian Fig (pink) that only associated with Carotene.

From this data it is derived that both green (Nepgen and Morado) and orange (Ofer and Gymno-Carpo) cultivars are associated with having more antioxidants (contents and capacity).

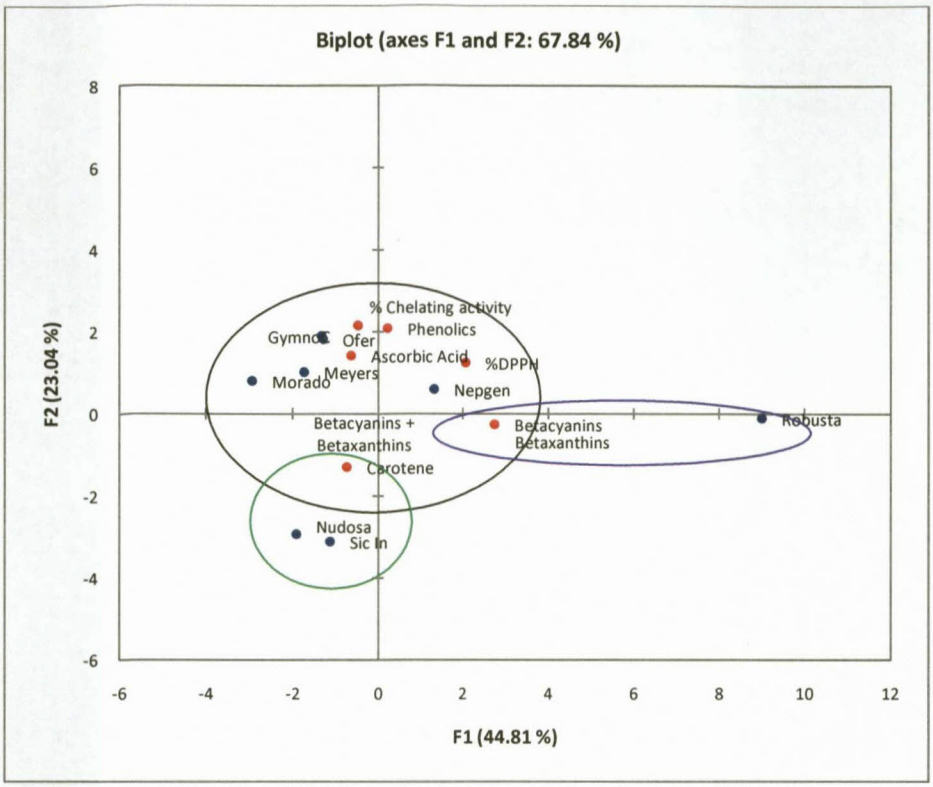


Figure 3.5: Principal component analysis of cultivar on the antioxidant properties of fresh cactus pear seed.

3.4.3.4 PCA of cultivar on the antioxidant properties of fresh cactus pear cladodes

In the PCA for cladodes there are very few close associations (68.27% explained) between cultivars and antioxidants. Only Robusta and Betalains are grouped together, while % Chelating activity, Ascorbic acid and Carotene are grouped together with Meyers (Figure 3.6). The other cultivars do not seem to associate with any of the antioxidants or antioxidant capacity tests in particular, indicating that cladodes do not have the strong associations with specific antioxidants as were the case in fruit, peel and seeds.

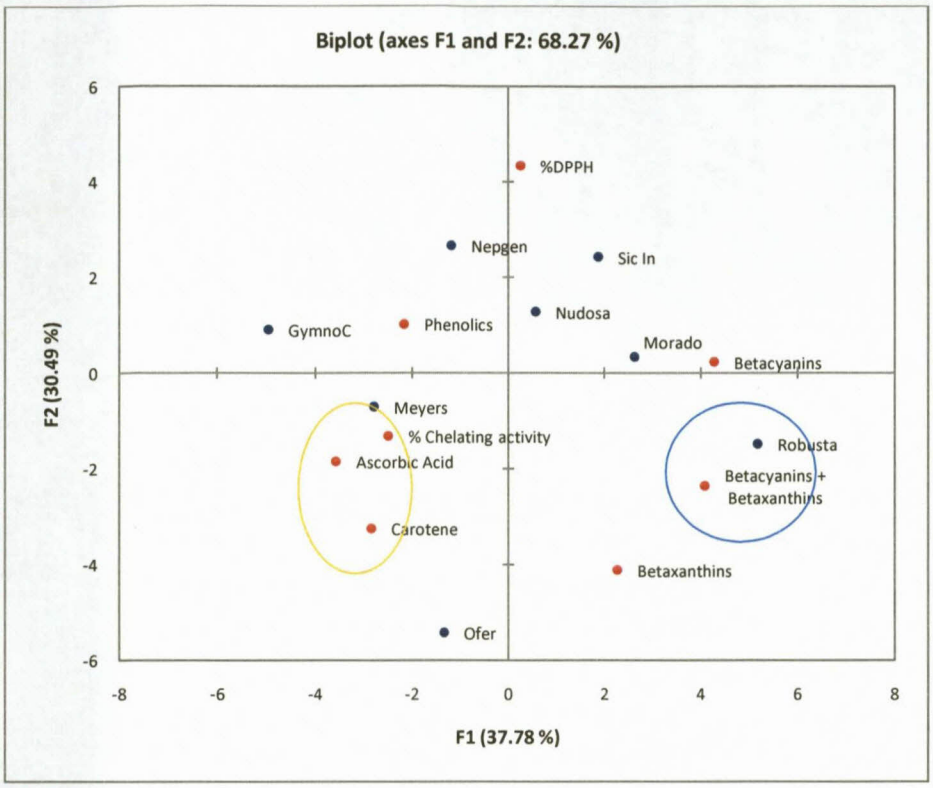


Figure 3.6: Principal component analysis of cultivar on the antioxidant properties of fresh cactus pear cladodes.

3.4.4 The effect of colour on the antioxidant properties of fresh cactus pear fruit (pulp)

One of the most important aims of the current study is to determine the influence of colour on antioxidant content and –capacity. Since colour is most evident in the fruit (pulp) itself, this part of the study only investigates the edible fruit of fresh cactus pears in order to link specific colour to eating quality. The effect of colour on the antioxidant potential and -content are indicated in Table 3.6. This was done by combining data of cultivars with the same colour fruit.

It was evident that purple fruit had the highest capacity (79.76%) to scavenge free radicals (significance level $p < 0.05$), and pink fruit the lowest (59.19%). The ability to chelate ferrous ions was the highest in purple (95.89%) and the lowest in orange (91.67%), although these were not significantly different. This finding is similar to data in studies done by Sumaya-Martinez *et al.* (2011), whose data agreed with Kuti (2004), that red and purple fruit had higher antiradical activity than other colours.

Table 3.6: The effect of colour on the antioxidant properties of fresh cactus pear fruit.

Colour	DPPH (n=3) %	Chelating activity (n=3) %	Phenolics (n=3) mg/kg GAE	Betacyanins (n=3) mg/kg	Betaxanthins (n=3) mg/kg	Betacyanins + Betaxanthins (n=3) mg/kg	Ascorbic Acid (n=3) mg/100g	Carotene (n=3) ug/g
Green	70.34 ± 16.21^{ab}	94.97 ± 1.60	12.59 ± 6.76	1.28 ± 0.71^{a}	0.89 ± 0.49^{a}	2.17 ± 1.20^{a}	51.90 ± 43.96^{ab}	0.72 ± 0.34^{a}
Orange	73.84 ± 5.93^{ab}	91.67 ± 6.06	17.74 ± 8.91	1.37 ± 0.28^{a}	0.96 ± 0.19^{a}	2.33 ± 0.47^{a}	94.67 ± 23.15^{b}	1.78 ± 0.23^{b}
Pink	59.19 ± 7.40^{a}	92.92 ± 4.59	9.57 ± 10.46	1.80 ± 1.02^{ab}	1.26 ± 0.71^{ab}	3.07 ± 1.73^{ab}	34.00 ± 20.65^{a}	1.20 ± 0.35^{ab}
Purple	79.76 ± 4.84^{b}	95.89 ± 0.94	10.66 ± 9.63	39.12 ± 46.63^{b}	27.38 ± 32.64^{b}	66.50 ± 79.28^{b}	32.60 ± 18.61^{a}	1.98 ± 1.14^{b}
AVG	70.78	93.86	12.64	10.89	7.62	18.52	53.29	1.42
STD	11.85	4.02	9.02	27.39	19.17	46.56	36.89	0.78
CV	16.73	4.28	71.37	251.49	251.49	251.49	69.21	54.65
Significance level	$p < 0.05$	NS	NS	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.01$	$p < 0.05$

Means with different superscripts in the same column differ significantly NS = Not significant

While orange fruit cultivars had the highest Phenolic (not significantly different) levels (17.74 mg/kg), pink fruit had the lowest values (9.57 mg/kg). Purple fruit had the highest Betalian content (66.5 mg/kg) (significance at $p < 0.05$) and green fruit the lowest (2.17 mg/kg).

As for Ascorbic acid the highest levels were for orange fruit (94.67 mg/100g) ($p < 0.01$) and the lowest for purple (32.6 mg/100g).

For Carotene, purple fruit once again showed its dominance (1.98 ug/g) ($p < 0.05$).

It may be concluded from this data that purple and orange fruit seem to be the best choice in terms of antioxidant content and capacity. Purple fruit had high % DPPH (79.76%), Chelating activity (95.89%), Carotene (1.98 ug/g) and Betalain (66.50 mg/kg) values. This finding correlates with a number of researchers who also found the highest antioxidant capacity in purple/red fruit (Kuti, 2004; Wang *et al.,* 1996; Fernández-López *et al.,* 2010; Sumaya-Martinez *et al.,* 2011). Orange fruit had high % DPPH (73.84%) readings but in contrast to purple fruit, it had higher Ascorbic acid (94.67 mg/100g) and Phenolic (17.74 mg/kg) content.

These findings mirror the conclusions in 3.4.3.1, 3.4.3.2 and 3.4.5, that purple fruit are associated with Betalains and Carotene, while orange fruit associate with Ascorbic acid and Phenolics. Though, both colours fruit have high antioxidant capacity.

3.4.5 Principal component analysis of colour on the antioxidant properties of fresh cactus fruit

In an attempt to identify the best colour regarding antioxidants, the effect of colour PCA was done. In Figure 3.7, the association between colour and antioxidants were plotted with 91.24% surety and it was found, in terms of colour, that green and pink fruit were only loosely associated with % Chelating activity, but with no other specific antioxidants or antioxidant properties. As was seen in the previous PCA's the purple fruit was associated with % DPPH, % Chelating activity, Betalains and Carotene, while orange fruit was grouped with Phenolics, Ascorbic acid, Carotene and % DPPH, as was seen for the PCA's before (Figure 3.3 and 3.4) and Table 3.6, these groups related every time.

From these statistics, the conclusion may be drawn that orange and purple fruit are more associated with antioxidant content and -capacity than green and pink fruit. Moreover, it can be stated that orange fruit derives its antioxidant capacity from Phenolics-, Ascorbic acid- and Carotene content combined, while with purple fruit antioxidant capacity is from the combined Betacyanin, Betaxanthin and Carotene content.

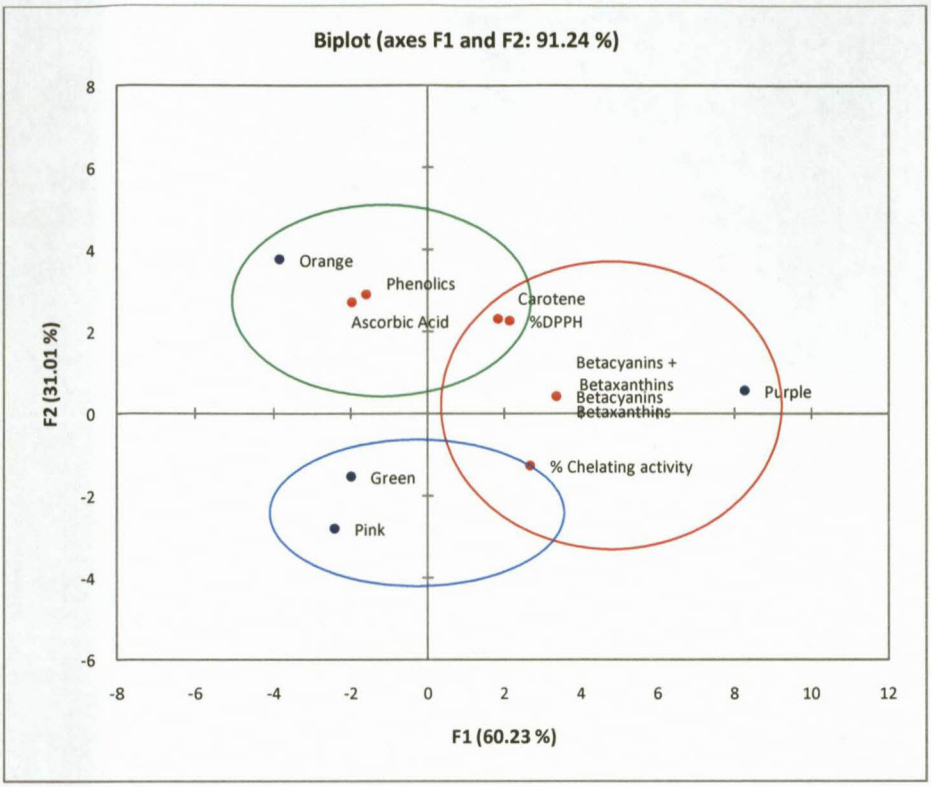


Figure 3.7: *Principal component analysis of colour on the antioxidant properties of fresh cactus pear fruit.*

3.4.6 Pearson correlation analysis

Pearson correlation analysis between the antioxidant properties of fruit, peel, seed and cladode tissue of fresh cactus pears was done (Table 3.7) to ascertain a measure of correlation strength between the individual antioxidant contents and antioxidant capacities (% DPPH and % Chelating activity of ferrous ions). A positive correlation value indicates that when one variable increases, the other will also increase, while a negative correlation indicates the opposite effect. Values > 0.5 indicate stronger correlations (important correlations are indicated in bold).

The Betaxanthins correlated strongly positively with Betacyanins (0.9945), meaning that both variables increase or decrease together in cactus pear fruit. This highly significant finding ($p < 0.001$) is consistent with the literature that both Betacyanin and Betaxanthin occur in cactus pear fruit while Betacyanin and Betaxanthin are mutually exclusive in red beetroot (Moreno-Alvarez *et al.*, 2008). It is also evident that the combined presence of Betacyanins and Betaxanthins (Betacyanine + Betaxanthin) is correlated to them individually (Betacyanine 0.9991 and Betaxanthin 0.9981) and the correlation is statistically highly significant ($p < 0.001$).

Table 3.7: Pearsons correlation analysis between the antioxidant properties of fruit, seed, peel and cladode tissue of fresh cactus pear.

	% DPPH	% Chelating activity	Phenolics	Betacyanins	Betaxanthin	Betacyanine + Betaxanthin	Ascorbic acid	Carotene
% DPPH	1.0000	**-0.3563*****	**-0.2286***	-0.3313**	-0.3254**	**-0.3293****	**0.0803**^{NS}	**-0.7272*****
% Chelating activity		1.0000	**-0.3475*****	0.0914^{NS}	0.0880^{NS}	0.0901^{NS}	0.0413^{NS}	0.0785^{NS}
Phenolics			1.0000	0.3201**	0.3389***	0.3282**	0.0344^{NS}	**0.5803*****
Betacyanins				1.0000	**0.9945*****	**0.9991*****	-0.1309^{NS}	**0.4565*****
Betaxanthin					1.0000	**0.9981*****	-0.1251^{NS}	**0.4594*****
Betacyanine + Betaxanthin						1.0000	-0.1287^{NS}	**0.4583*****
Ascorbic acid							1.0000	-0.0247^{NS}
Carotene								1.0000

NS = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

There was a moderate negative correlation between Carotene and % DPPH (-0.7272) which indicated that as % DPPH increased, the Carotene content decreased. This indicated that Carotene did not play an important role in contributing to the antioxidant capacity in cactus pear fruit as Carotene had very low values in the fruit and peel. The correlation measured show a slight (but significant) positive correlation between Phenolics and Carotene (0.5803) (this correlation was also seen Figure 3.3). An even weaker correlation between Phenolics and the combined Betalains was reported (0.4583). The context of data should be understood to evaluate results of a Pearson Correlation Analysis, therefore the very slight negative correlation between % Chelating activity and % DPPH is noteworthy since it indicated that as the one antioxidant capacity measure increased the other decreased. It is also noteworthy that both the % DPPH and % Chelating activity have slight but significantly negative correlations with Phenolics (-0.2286 and -0.3313) and % DPPH with Betalains (-0.3293), no correlation (not significant) with Ascorbic acid (0.0803) and only a moderately negative correlation with Carotene (-0.7272). The specific compound that contributes mostly toward the antioxidant capacity is not indicated in this correlation. The conclusion could be made that another compound, not tested in these studies may be responsible for antioxidant capacity, or alternatively that all the antioxidant compounds work synergistically to contribute towards the exceptionally high antioxidant potential found in cactus pear fruit plants.

There are well known (Vitamin C, E and A) and lesser known vitamins that are believed to work synergistically (Gülçin, 2012). Kuti (2004) suggested synergistic effect while other authors reported correlations between colour of fruit and antioxidant content (Butera *et al.*, 2002; Stintzing *et al.*, 2005; Sumaya-Martinez *et al.*, 2011).

3.4.7 The interaction between cultivar, tissue type and colour on the antioxidant properties in fresh cactus pears.

The following table (Table 3.8) shows the interaction between cultivar, tissue type and colour on the antioxidant properties of cactus pear. In order to investigate this complex multi layered issue it was necessary to separate the antioxidant contents and –potential data and to display it in a graphical format. The same data was used for the graphics (Figures 3.8 to 3.14) as was indicated in the table (Table 3.8). The table is included since it gives the complete picture across antioxidants, products, colour and tissue type, with its necessary averages and statistical analysis.

Table 3.8: The effect of cultivar and tissue type on the antioxidant properties of fresh cactus pear.

Cultivar	Tissue	DPPH (n=3) %	Chelating activity (n=3) %	Phenolics (n=3) mg/kg GAE	Betacyanins (n=3) mg/kg	Betaxanthins (n=3) mg/kg	Betacyanins + Betaxanthins (n=3) mg/kg	Ascorbic Acid (n=3) mg/100g	Carotene (n=3) ug/g
Nepgen (Green)	Fruit	83.63 ± 1.30^{ef}	94.94 ± 0.37^{ef}	16.72 ± 7.48^{ab}	1.83 ± 0.55^{a}	1.28 ± 0.39^{a}	3.11 ± 0.94^{a}	91.69 ± 8.98^{fgh}	0.62 ± 0.19^{a}
	Peel	91.67 ± 1.04^{ef}	81.67 ± 5.20^{bcdef}	15.96 ± 8.06^{ab}	0.89 ± 0.37^{a}	0.62 ± 0.26^{a}	1.52 ± 0.63^{a}	55.88 ± 7.71^{abcdefgh}	3.46 ± 0.40^{abc}
	Seed	42.35 ± 9.13^{ab}	94.17 ± 1.44^{ef}	184.30 ± 31.76^{bcdef}	69.89 ± 33.60^{cd}	48.92 ± 23.52^{cd}	118.81 ± 57.13^{cd}	28.98 ± 0.36^{abcd}	40.15 ± 2.84^{e}
	Cladode	94.49 ± 1.37^{f}	89.17 ± 1.44^{def}	241.53 ± 32.75^{def}	4.74 ± 0.39^{ab}	3.32 ± 0.27^{ab}	8.07 ± 0.66^{ab}	26.78 ± 1.01^{abc}	6.72 ± 1.50^{abc}
Meyers (Pink)	Fruit	58.65 ± 11.49^{bcd}	93.33 ± 5.20^{ef}	17.45 ± 9.15^{ab}	2.71 ± 0.15^{ab}	1.90 ± 0.10^{ab}	4.62 ± 0.25^{ab}	52.52 ± 5.75^{abcdefgh}	0.92 ± 0.16^{a}
	Peel	96.25 ± 2.91^{f}	70.00 ± 10.90^{bc}	58.88 ± 25.60^{abc}	6.87 ± 0.75^{ab}	4.81 ± 0.53^{ab}	11.69 ± 1.28^{ab}	86.28 ± 21.90^{efgh}	1.79 ± 0.35^{a}
	Seed	40.20 ± 12.99^{ab}	90.00 ± 5.00^{def}	276.63 ± 76.42^{ef}	39.01 ± 14.50^{abcd}	27.31 ± 10.15^{abcd}	66.32 ± 24.66^{abcd}	83.22 ± 22.72^{defgh}	66.54 ± 13.08^{f}
	Cladode	92.74 ± 3.27^{ef}	80.83 ± 2.89^{bcdef}	257.25 ± 60.00^{ef}	7.54 ± 1.97^{ab}	5.28 ± 1.38^{ab}	12.82 ± 3.34^{ab}	67.25 ± 18.65^{bcdefgh}	18.15 ± 7.20^{cd}
Ofer (Orange)	Fruit	77.56 ± 4.61^{def}	94.17 ± 3.82^{ef}	13.41 ± 1.62^{a}	1.20 ± 0.17^{a}	0.84 ± 0.12^{a}	2.05 ± 0.28^{a}	94.07 ± 29.97^{h}	1.69 ± 0.32^{a}
	Peel	93.85 ± 0.75^{f}	69.17 ± 3.82^{b}	21.31 ± 14.73^{ab}	1.11 ± 0.22^{a}	0.78 ± 0.15^{a}	1.89 ± 0.37^{a}	64.24 ± 16.73^{abcdefgh}	4.80 ± 0.59^{abc}
	Seed	39.25 ± 10.14^{ab}	88.33 ± 3.82^{cdef}	258.83 ± 30.32^{ef}	35.58 ± 17.16^{abcd}	24.91 ± 12.01^{abcd}	60.49 ± 29.17^{abcd}	86.75 ± 36.14^{fgh}	32.59 ± 9.84^{de}
	Cladode	83.77 ± 10.41^{ef}	80.83 ± 7.22^{bcdef}	239.47 ± 17.12^{def}	3.17 ± 2.24^{ab}	16.98 ± 1.26^{abcd}	20.15 ± 2.78^{ab}	58.74 ± 11.14^{abcdefgh}	17.87 ± 1.30^{bcd}
Gymno C (Orange)	Fruit	70.12 ± 4.99^{cde}	89.17 ± 7.64^{def}	22.08 ± 11.81^{ab}	1.54 ± 0.28^{a}	1.08 ± 0.20^{a}	2.62 ± 0.48^{a}	95.27 ± 21.00^{h}	1.86 ± 0.09^{a}
	Peel	91.18 ± 2.11^{ef}	72.50 ± 9.01^{bcd}	14.04 ± 2.48^{ab}	2.21 ± 0.21^{a}	1.55 ± 0.15^{a}	3.75 ± 0.37^{a}	68.04 ± 38.66^{bcdefgh}	3.99 ± 1.32^{abc}
	Seed	48.64 ± 12.36^{abc}	90.83 ± 5.20^{def}	278.73 ± 55.34^{ef}	22.41 ± 3.75^{abcd}	15.69 ± 2.63^{abcd}	38.10 ± 6.38^{abcd}	49.91 ± 17.54^{abcdefgh}	43.31 ± 13.74^{e}
	Cladode	95.53 ± 0.15^{f}	77.50 ± 2.50^{bcde}	270.93 ± 117.99^{ef}	3.17 ± 2.24^{ab}	2.22 ± 1.56^{ab}	5.40 ± 3.80^{ab}	77.40 ± 11.34^{cdefgh}	17.87 ± 1.30^{bcd}
Robusta (Purple)	Fruit	77.05 ± 4.53^{def}	95.09 ± 0.38^{ef}	18.47 ± 6.02^{ab}	74.47 ± 41.06^{d}	52.13 ± 28.74^{d}	126.60 ± 69.81^{d}	32.38 ± 22.99^{abcde}	1.58 ± 0.09^{a}
	Peel	91.65 ± 0.44^{ef}	97.32 ± 0.38^{f}	7.44 ± 4.48^{a}	42.62 ± 8.79^{abcd}	29.84 ± 6.15^{abcd}	72.46 ± 14.94^{abcd}	61.16 ± 25.48^{abcdefgh}	6.06 ± 2.83^{abc}
	Seed	56.50 ± 17.85^{bcd}	85.83 ± 6.29^{bcdef}	258.35 ± 168.32^{ef}	160.88 ± 69.85^{e}	112.62 ± 48.89^{e}	273.50 ± 118.74^{e}	36.12 ± 10.64^{abcdef}	41.67 ± 6.17^{e}
	Cladode	92.50 ± 0.91^{ef}	70.00 ± 9.01^{bc}	42.84 ± 18.92^{abc}	17.18 ± 7.74^{abc}	12.02 ± 5.42^{abc}	29.20 ± 13.16^{abc}	24.04 ± 7.44^{abc}	11.29 ± 3.04^{abc}
Morado (Green)	Fruit	57.05 ± 11.19^{bcd}	95.00 ± 2.50^{ef}	8.46 ± 2.66^{a}	0.72 ± 0.14^{a}	0.51 ± 0.10^{a}	1.23 ± 0.25^{a}	12.11 ± 0.24^{a}	0.82 ± 0.48^{a}
	Peel	91.70 ± 2.20^{ef}	87.50 ± 9.01^{bcdef}	31.88 ± 18.22^{ab}	1.84 ± 0.73^{a}	1.29 ± 0.51^{a}	3.13 ± 1.23^{a}	29.23 ± 3.89^{abcd}	3.59 ± 0.92^{abc}
	Seed	32.39 ± 6.24^{a}	91.67 ± 1.44^{ef}	291.46 ± 70.86^{ef}	17.48 ± 6.55^{abc}	12.24 ± 4.59^{abc}	29.72 ± 11.14^{abc}	29.72 ± 1.12^{abcd}	47.04 ± 2.12^{e}
	Cladode	95.31 ± 0.15^{f}	40.77 ± 1.33^{a}	324.87 ± 20.94^{f}	14.82 ± 4.84^{ab}	10.37 ± 3.39^{ab}	25.20 ± 8.23^{ab}	47.78 ± 11.52^{abcdefgh}	11.58 ± 0.85^{abc}
Sic In Fig (Pink)	Fruit	59.73 ± 2.04^{bcd}	92.50 ± 5.00^{ef}	1.68 ± 1.79^{a}	0.89 ± 0.27^{a}	0.62 ± 0.19^{a}	1.51 ± 0.46^{a}	15.47 ± 1.92^{ab}	1.47 ± 0.25^{a}
	Peel	90.95 ± 8.41^{ef}	87.50 ± 2.50^{bcdef}	12.56 ± 2.04^{a}	3.23 ± 0.27^{ab}	2.26 ± 0.19^{ab}	5.49 ± 0.47^{ab}	24.22 ± 5.89^{abc}	2.11 ± 0.14^{a}
	Seed	26.68 ± 11.01^{a}	83.33 ± 18.09^{bcdef}	204.47 ± 88.92^{cdef}	56.50 ± 19.02^{bcd}	39.55 ± 13.32^{bcd}	96.04 ± 32.34^{bcd}	39.68 ± 15.87^{abcdefg}	69.24 ± 3.72^{f}
	Cladode	94.08 ± 0.93^{f}	27.44 ± 4.44^{a}	304.41 ± 96.43^{f}	9.19 ± 1.91^{ab}	6.44 ± 1.34^{ab}	15.63 ± 3.24^{ab}	27.31 ± 10.21^{abc}	7.29 ± 0.51^{abc}
Nudosa (Purple)	Fruit	82.47 ± 4.00^{ef}	96.68 ± 0.42^{f}	2.85 ± 3.53^{a}	3.76 ± 1.01^{ab}	2.64 ± 0.71^{ab}	6.40 ± 1.72^{ab}	32.82 ± 18.37^{abcdef}	2.38 ± 1.66^{ab}
	Peel	96.85 ± 2.18^{f}	96.63 ± 0.13^{f}	10.59 ± 1.40^{a}	5.08 ± 1.55^{ab}	3.55 ± 1.09^{ab}	8.63 ± 2.64^{ab}	46.40 ± 11.47^{abcdefgh}	2.01 ± 0.58^{a}
	Seed	37.86 ± 4.66^{ab}	84.17 ± 3.82^{bcdef}	74.85 ± 75.97^{abcd}	23.86 ± 8.33^{abcd}	16.70 ± 5.83^{abcd}	40.56 ± 14.16^{abcd}	28.22 ± 1.30^{abc}	45.62 ± 12.32^{e}
	Cladode	93.91 ± 0.38^{f}	80.26 ± 1.94^{bcdef}	131.32 ± 23.48^{abcde}	7.59 ± 0.70^{ab}	5.31 ± 0.49^{ab}	12.90 ± 1.19^{ab}	19.15 ± 12.43^{ab}	10.70 ± 1.08^{abc}
AVG		74.27	83.38	122.31	20.13	14.55	34.67	49.78	16.46
STD		23.45	15.97	127.51	35.24	24.58	59.73	28.64	20.42
CV		31.57	19.15	104.25	175.09	168.92	172.27	57.54	124.06
Significance level		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

Means with different superscripts in the same column differ significantly

3.4.7.1 Betalains

Both betacyanin and betaxanthin are bioavailable compounds that behave as scavengers of biologically relevant radicals (Livrea & Tesoriere, 2006). Betalains carry a phenolic and an acyclic amine group, which are excellent electron donors and are able to stabilize radicals. The consumption of cactus pear fruit positively affects the body's redox balance and decrease the oxidative damage of lipids as a result of the betalain content in the fruit (Moreno-Alvarez *et al.*, 2008).

A. Betacyanins

The levels of Betacyanins were not significantly different ($p < 0.001$) in cultivars, colours and tissue types except for Nepgen seeds, Sicilian Indian Fig seeds and Robusta fruit and seeds which had significantly higher levels of Betacyanins (Figure 3.8).

The Betacyanin level in Robusta was the highest in general, especially in fruit. Peel and seeds were higher than any of the other cultivars, whereas the cladode levels compared well to other cultivars.

Purple cultivars (specifically Robusta) had the highest Betacyanins while green and orange fruit had the lowest values. The pink and other purple fruit tested, namely the Meyers, Sicilian Indian Fig (pink) and Nudosa (purple), had slightly elevated levels of Betacyanins in the fruit and peel, but it is very interesting that these cultivars did not have significantly more Betacyanins than the green and orange fruit and peel even though they appear pink in colour. As Betacyanins are purple coloured pigments, it may be expected that it would dominate only in the purple fruit, but it is evident that the seeds of all cultivars had elevated (not significant) levels.

When total contribution of all the tissue types of different cultivars toward Betacyanin content is considered, Robusta stood out as the best contributor, with Sicilian Indian Fig a distant second. Overall, the highest levels were found in seeds (as expected as seed samples were dried). Cladodes are a better source than fruit and peel in all the cultivars, except for Robusta.

These results agree with results from previous studies with the notion that purple cactus pear fruit contains very high levels of Betalains whereas pink, yellow and orange cultivars contains lower amounts. In this study the levels in purple fruit were higher than in Castellanos-Santiago and Yahia (2008) (5.29 mg/g) and lower than in Sumaya-Martínez *et al.* (2005) (333 mg/l) and Stintzing *et al.* (2005) (486.7 mg/kg). According to Castellanos-Santiago and Yahia (2008) the purple robusta-type fruit studied contained similar amounts to red beetroot (40-60 mg/100 g or

71-77 mg/100 g), but in these results, it is clear that Robusta contains ten times less betacyanins than red beetroot (7.5 mg/100 g). No literature is available on the Betalain content in seeds and cladodes.

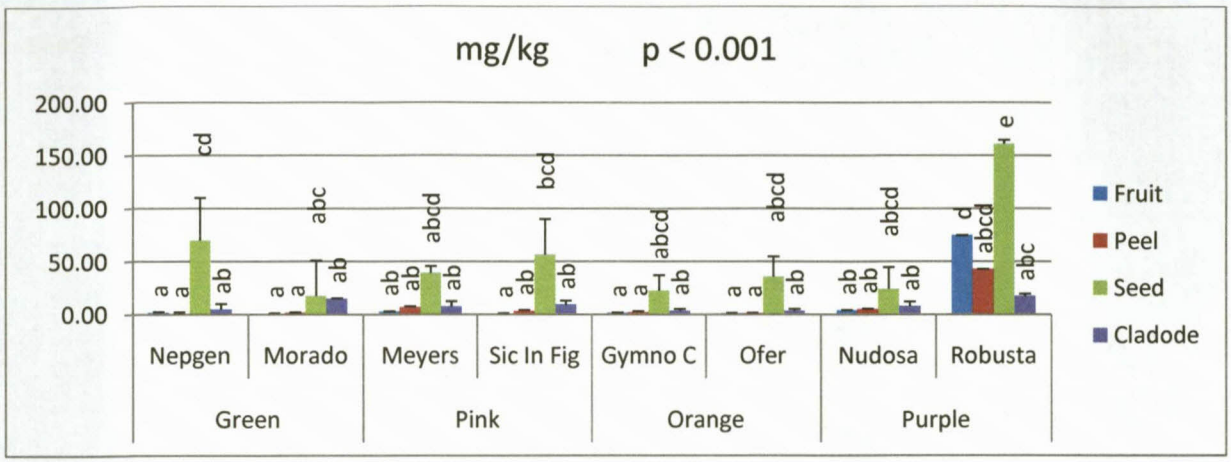


Figure 3.8: The effect of colour, cultivar and tissue type on Betacyanins

B. Betaxanthins

In general, Robusta had the highest values Betaxanthins (as was seen for Betacyanins as well) for all tissue types (Figure 3.9).

Purple had the highest values, with pink slightly higher than green and orange (Figure 3.9). These findings are noteworthy as the orange coloured fruit did not show higher levels of Betaxanthins than the other coloured fruit even though Betaxanthins are yellow pigments. It can be derived from this data that the orange colour in cactus pears is not as a result of Betaxanthin content. Although, Nudosa (purple) and Meyers (pink) had slightly more Betalains (Betacyanins + Betaxanthins) than other cultivar fruit and thus it could be speculated that it could provide the fruit with enough purple pigment for it to appear pink/purple.

Seeds had the highest levels overall and cladodes had higher values than fruit and peel. The purple fruit and seeds from Robusta, together with the seeds from Sicilian Indian Fig and Nepgen, had significantly more Betaxanthins (yellow pigment) than the other tissue types. Robusta cladodes did not have significantly more Betaxanthins than cladodes from other cultivars.

It is therefore concluded that betacyanins and betaxanthin contents are linear, meaning that cactus fruit with high levels of Betacyanins also have high levels of Betaxanthins. This agrees

with the literature. The levels of Betacyanins found in cactus pear fruit in the current study were higher than in Castellanos-Santiago and Yahia (2008) (2.86 mg/g) and lower than in Sumaya-Martínez *et al.* (2005) (147 mg/l) and Stintzing *et al.* (2005) (553.7 mg/kg).

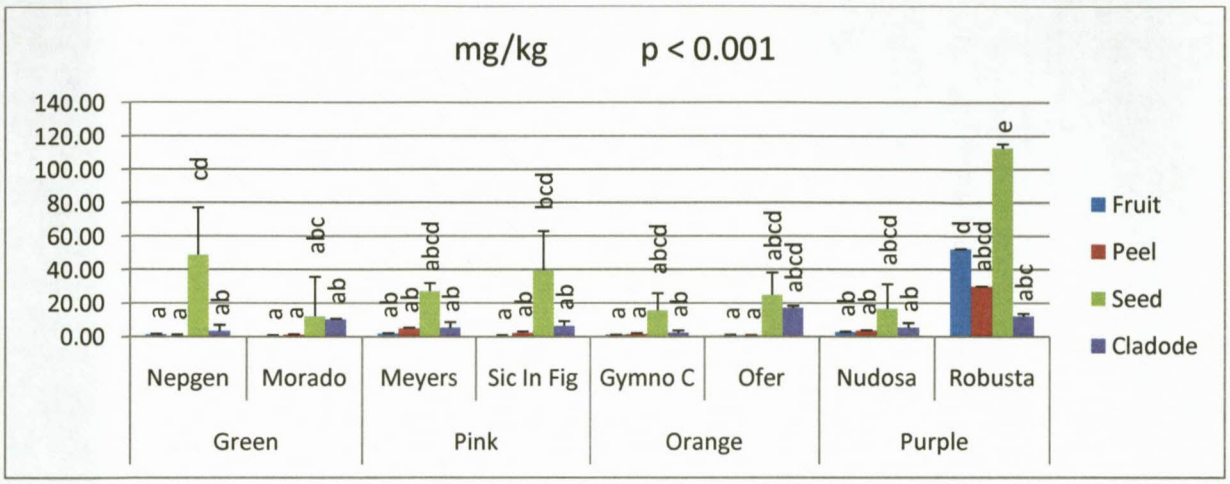


Figure 3.9: The effect of colour, cultivar and tissue type on Betaxanthins

3.4.7.2 Ascorbic acid

Ascorbic acid is one of the most powerful natural antioxidants and the least toxic. It is a special antioxidant because it can transfer a single electron and can successfully reduce a reactive compound (Gülçin, 2012). Ascorbic acid content in cactus pear fruit positively affected the body's redox balance, decreased lipid oxidation and improved antioxidant status of healthy humans (Livrea & Tesoriere, 2006).

It is known that cactus pear fruit has significant amounts of Ascorbic acid (Sáenz, 2000; Moβhammer *et al.*, 2006a). In general Nepgen, Meyers, Gymno-Carpo and Ofer appear to have the highest (> 50 mg/kg) Ascorbic acid content (Figure 3.10). None of the measurements, across colours, cultivars and tissue types differed significantly, but from the data it is evident that Ofer (94.07 mg/100 g) (orange fruit) had the highest and Morado (12.11 mg/100 g) (green fruit) the lowest levels of Ascorbic acid.

Orange and pink had the highest values. Nepgen (green) showed very high levels as well, whereas the other green cultivar (Morado) had the lowest levels of ascorbic acid. Therefore it may be stated that orange/yellow colour in cactus pear fruit may indicate high content of Ascorbic acid but other colours cannot be excluded from having high contents.

The content of Ascorbic acid is not correlated in the tissue types as seen in Figure 3.10. From these results it can be concluded that both the pulp and peel of Nepgen, Ofer and Gymno-Carpo are rich in ascorbic acid, but when the total contribution of pulp, peel, seeds and cladodes are taken into account, Meyers is equal to both orange cultivars (Ofer and Gymno-Carpo).

The levels of Ascorbic acid found in this study were on average (49.78 mg/100 g) higher than that found by other researchers (Kuti, 2004; Stintzing *et al.,* 2005; Figueroa-Cares *et al.,* 2010; Fernández-López *et al.,* 2010; De Wit *et al.,* 2010; Yahia and Mondragon-Jacobo, 2011; Sumaya-Martínez *et al.,* 2011; Coria Cayapán *et al.,* 2011). In this study Ascorbic acid levels in fresh fruit and processed products ranged from 12 to 95 mg/100 g whereas other researchers presented levels from 0.79 mg/100 g (Kuti, 2004) to 48 mg/100 g (Coria-Cayapán *et al.*, 2011). The conclusion may be made that cactus pear is higher in Ascorbic acid than most other common fruits, such as apple, pear, grape and banana (Sáenz, 2000) and show values that are typical for citrus fruit, mangoes and guavas (Stintzing *et al.,* 2005).

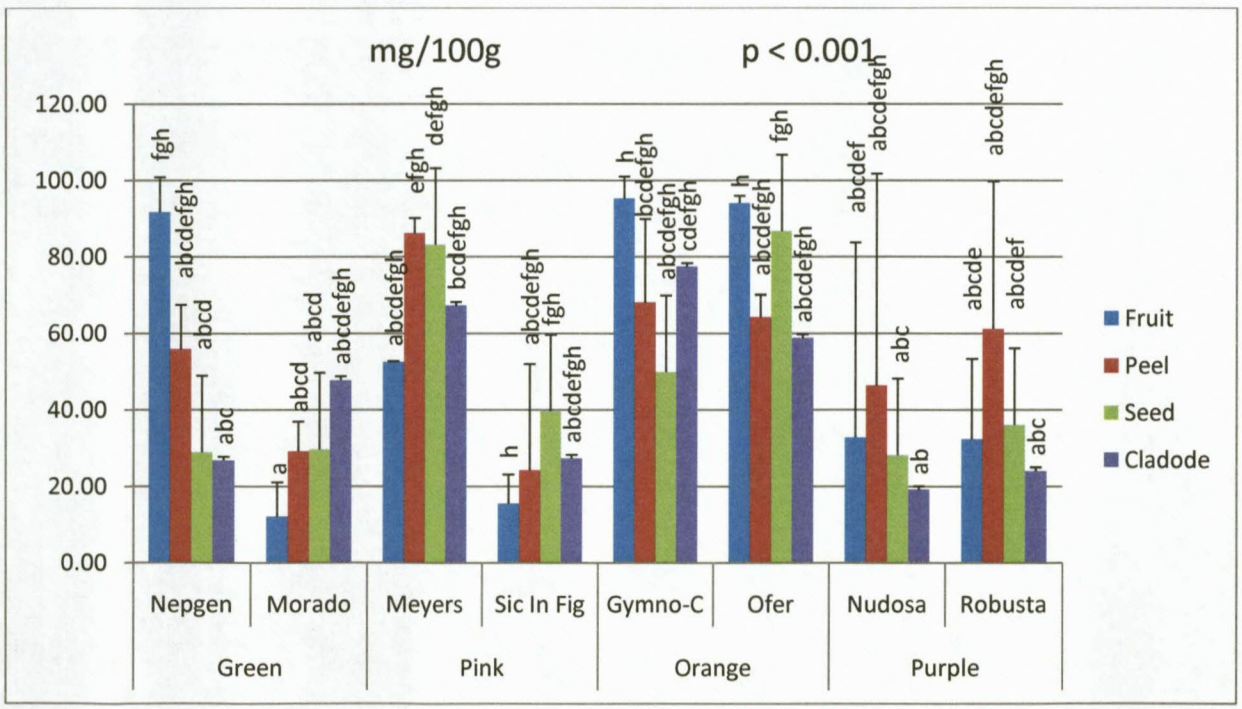


Figure 3.10: The effect of colour, cultivar and tissue type on Ascorbic acid.

3.4.7.3 Carotene

High intakes of carotenoid rich vegetables and fruits and high blood levels of β-carotene are associated with decreased incidence of some cancers (Stahl & Sies, 2003).

Very low levels were detected in fruit and peel in all cultivars; in fact, the highest levels were in seeds. Sicilian Indian Fig and Meyers had the highest contents in seeds (69.24 ug/g and 66.54 ug/g respectively) (Figure 3.11). For Carotene content there was no specific cultivar that stood out as one with the highest levels, but there were very specific trends in terms of colour.

The orange cultivars did not contain the most Carotene as was expected with orange coloured fruit; in fact, the pink cultivars contained the highest levels. In previous studies quite a few researchers found very little carotene in cactus pear fruit in general (0.021 – 17.7 µg/g) but found that some pink and purple fruit had higher Carotene contents than orange, yellow and white fruit (Tesoriere *et al.,* 2005; Morales & Saenz, 2009; Figueroa-Cares *et al.*, 2010 and Yahia & Mondragon-Jacobo, 2011). Results from this study correlated with findings from other studies in the sense that orange fruit did not appear to have the most Carotene. Pink fruit had the highest levels with purple fruit following closely; purple fruit had similar amounts than orange cultivars. It may be derived from the data that fruit that are high in Betalain content (Figure 3.8 & 3.9) are also high in Carotene content. It was explained by Yahia and Mondargon-Jacobo (2011) who also found that fruit with high Betalains also appear to have high Carotene levels, that the pink and purple pigments (Betacyanins) overpower the yellow colour of Carotene and therefore the fruit appears pink/purple in colour.

The cladodes on average contained more Carotene than fruit and peel but the levels were not statistically different. When other researchers (Jaramillo-Flores *et al.,* 2003; Medina-Torres *et al.*, 2005; Bensadón *et al.*, 2010) studied cladodes for Carotene content, they found different amounts (0.02 ug/g to 231.8 ug/g), but nevertheless, it was considerably higher levels than what was found in the fruit. There were no significant differences between different colours or cultivars, thus the finding by Bensadón *et al.* (2010) that Carotenoid content in cladodes does not depend on colour or cultivar is agreed with. Medina-Torres *et al.* (2011) compared cladodes to other vegetables and found that it has a higher carotenoid content than baby carrots, beetroot, spinach and lettuce.

In terms of tissue type, the results showed that the seeds of the all the cultivars differed statistically from the fruit, peel and the cladodes and had between 32 and 69 times more carotene than the fruit (Figure 3.11). The Carotene contents of the fruit (pulp and peel) in this study were low compared to seeds and cladodes (Figure 3.11) and thus it can be concluded that cactus pear fruit is not a good source of Carotene, but that the seeds has a high content that would most probably be present in the oily fraction of the seed.

Sicilian Indian Fig demonstrated the highest levels of Carotene in the seeds, but Meyers had the most Carotene when all the tissue types were considered. No literature dealing with Carotene content in cactus fruit seeds are available for comparative purposes.

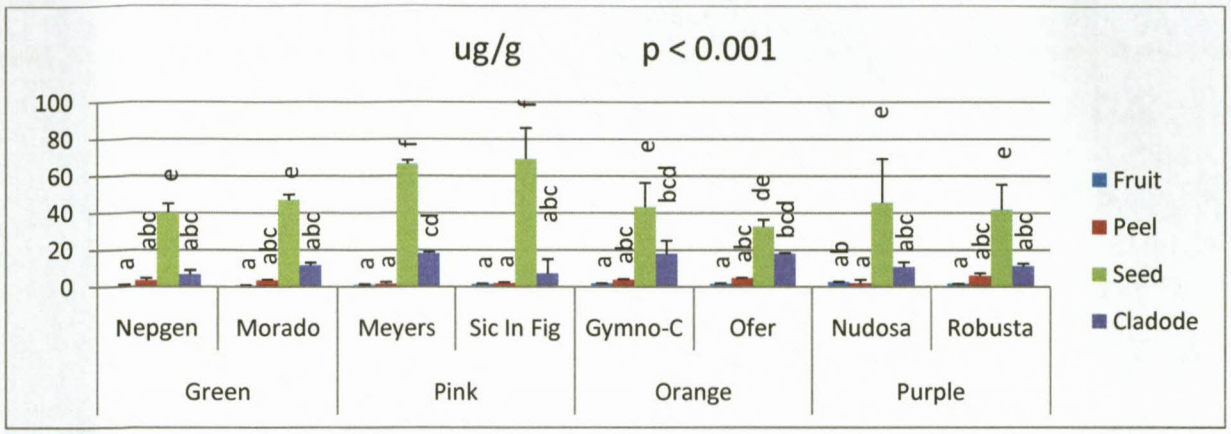


Figure 3.11: The effect of colour, cultivar and tissue type on Carotene content

3.4.7.4 Phenolics

Epidemiological studies show that consumption of fruit and vegetables with high Phenolic content correlate with reduced cardio- and cerebrovascular diseases and cancer mortality. Phenolic compounds may produce their beneficial effects by scavenging free radicals. In the past few years there has been an increasing interest in determining relevant dietary sources of Phenolics (Gil *et al.*, 2002).

The statistical similarities in low fruit- and peel contents and high seed- and cladode contents were the only correlations seen in these findings (Figure 3.12); the colour and cultivar does not seem to have an effect on Phenolic content. Morado, Meyers, Sicilian Indian Fig, Gymno-Carpo and Ofer had the highest levels, although there were no significant differences amongst fruit and peel, together with the seeds and cladodes they had levels above 500 mg/kg (Figure 3.12).

In many previous studies it was found that purple or red fruit yielded the most Phenolics (Kuti, 2004; Stintzing *et al.*, 2005; Chavez-Santoscoy *et al.*, 2009; Morales & Sáenz, 2009; Alimi *et al.*, 2012, Yahia & Mondragon-Jacobo, 2011 and Coria-Cayupán *et al.*, 2011), that result was not found in this study as there were no significant differences in Phenolics in the fruit or peel of pink/purple fruit. In fact Gymno-Carpo (orange) fruit had the highest readings in fruit (22.08 mg/kg) and Meyers (pink) in peel (58.88 mg/kg). It seems as if, in general, Robusta (purple) had average levels in fruit (18.47 mg/kg), the lowest levels in peel (7.44 mg/kg) and statistically significant low levels in cladodes (42.84 mg/kg).

In previous studies, Phenolics content in cactus pear fruit ranged from 21.88 – 746 mg/kg (Galati *et al.*, 2003; Fernández-López *et al.*, 2010) but the range for fruit and peel was lower (1.68 – 58 mg/kg) in this study. However, the seeds and cladodes would be very good sources of total Phenolics (Figure 3.12). The Phenolic levels for cladodes in this study correlated with data from Santos-Zea *et al.* (2010) who found a minimum of 318.1 mg/kg and Medina Torres *et al.* (2011) who found 1 g/kg (1000 mg/kg) in the cladodes of Mexican cactus pears. In this study the levels for cladodes fluctuated from 42.84 to 324 mg/kg. There were no statistically different levels between seeds and cladodes except for Robusta and Nudosa (both purple cultivars) where the cladodes had statistically significant lower contents of Phenolics.

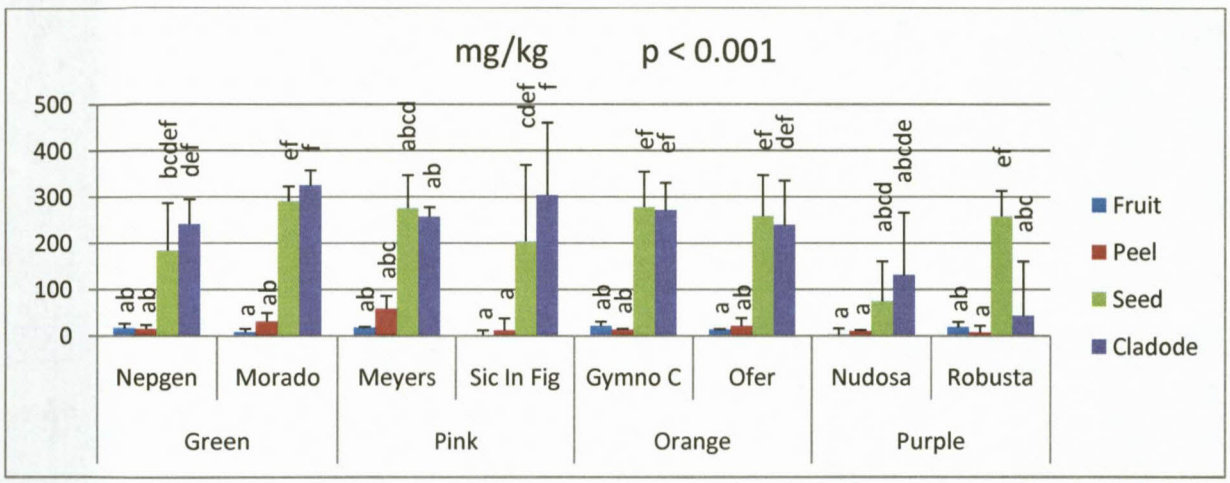


Figure 3.12: The effect of colour, cultivar and tissue type on Phenolic content

3.4.7.5 % DPPH

The DPPH scavenging assay relies on the principle that radical scavengers may directly react with and scavenge peroxide radicals in order to terminate the peroxidation chain reactions and improve the quality and stability of food products (Gülçin, 2012).

There were statistically significant differences in terms of radical scavenging ability (% DPPH) amongst cultivars. Statistically, fruit from Morado (green), Meyers and Sicilian Indian Fig (both pink) had significantly lower radical scavenging capacity (Figure 3.13), but it is evident that these cultivars do not lack an overall radical scavenging capacity when the fruit, peel, seeds and cladodes are considered.

The radical scavenging ability of the combined antioxidants in cactus pears was significantly similar between the different tissue types (Figure 3.13). The antioxidant capacity of peel, seeds and cladodes demonstrated similar trends amongst cultivars, but were significantly different

among fruit. These differences among fruit may be attributed to the highly significant differences between Cultivar x Tissue type interaction as indicated in the ANOVA analysis (Table 3.5).

The peel and cladodes demonstrated exceptionally high radical scavenging ability, the cladodes had readings between 83 and 95% and the peel 90 to 96%. These results indicate that peels should be included where possible, such as in the making of juice, to increase the antioxidant capacity of the product.

In terms of colour, these findings agreed with findings by Chavez-Santoscoy *et al.* (2009), Figueroa-Cares *et al.* (2010) and Yahia and Mondragon-Jacobo (2011) that all cactus pear fruit had high antioxidant capacity despite their colour and in fact, instead of differences amongst colour, there were significant differences in the individual cultivars and tissue types in terms of antioxidant contents.

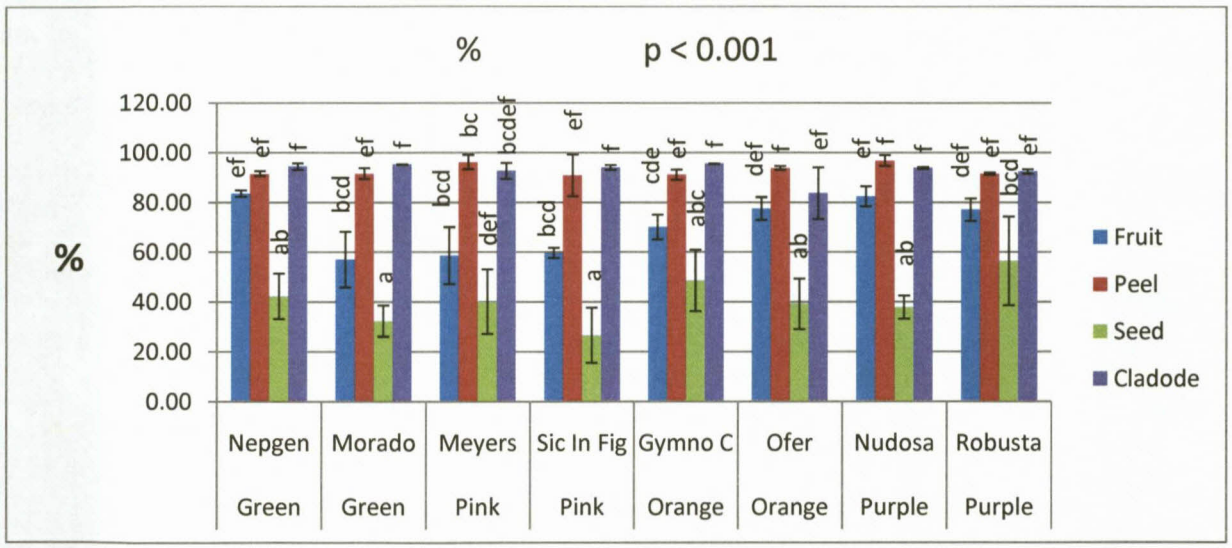


Figure 3.13: The effect of colour, cultivar and tissue type on DPPH capacity

3.4.7.6 % Chelating activity of ferrous ions

An effective ferrous ion chelator could offer protection to human cells by removing iron that may otherwise participate in $HO^{\cdot}$ generation. The chelating ability of antioxidants may be an alternative but effective way of preventing oxidative stress and therefore several diseases (Gülçin, 2012).

Among, cultivars, colours and tissue types, the chelating activity was generally high (average 83.38%) and mostly not significantly different (Figure 3.14). Nevertheless, it appears as if Nepgen, Meyers, Gymno-Carpo, Ofer and Nudosa had the best ability to chelate ferrous ions.

The cladodes from Morado and Sicilian Indian Fig and the peel from Meyers, Ofer and Gymno-Carpo had significantly lower values.

Regarding colour, orange and purple seem to contribute more than green and pink cultivars (Figure 3.14). The readings that were statistically the highest, were the peel (97.32%) from Robusta (purple) and both the fruit (96.68%) and peel (96.63%) from Nudosa (purple). The lowest readings were Morado (40.77%) (green) and Sicilian Indian Fig (27.44%) (pink).

Chelating activity of fruit was higher than other tissue types with levels ranging from 89.17% (Gymno-Carpo) to 96.68% (Nudosa) as seen in Figure 3.14. Peel and cladodes had the lowest levels with levels from 69.17% (Ofer) to 97.32% (Robusta) for peel and 27.44% (Sicilian Indian Fig) to 89.17% (Nepgen) for cladodes. Peel values for Meyers, Ofer and Gymno-Carpo were significantly lower. In contrast to the % DPPH findings in 3.4.7.5 regarding tissue type, where fruit had inconsistent readings, the fruit was very consistent in their excellent ability to chelate ferrous ions (average 93.86%) and cladodes were not consistent amongst cultivars.

When Sumaya-Martinez *et al.* (2011) determined the chelating activity of different coloured cactus pear fruits, it was concluded that it does not depend on colour of cactus pear since activity did not significantly differ in all studied cultivars. In this study the same results were found for fruit, peel and seeds. The reason for cladodes not to correspond with these results cannot be explained as it does not correlate with any individual antioxidant component (as seen in 3.4.3.4).

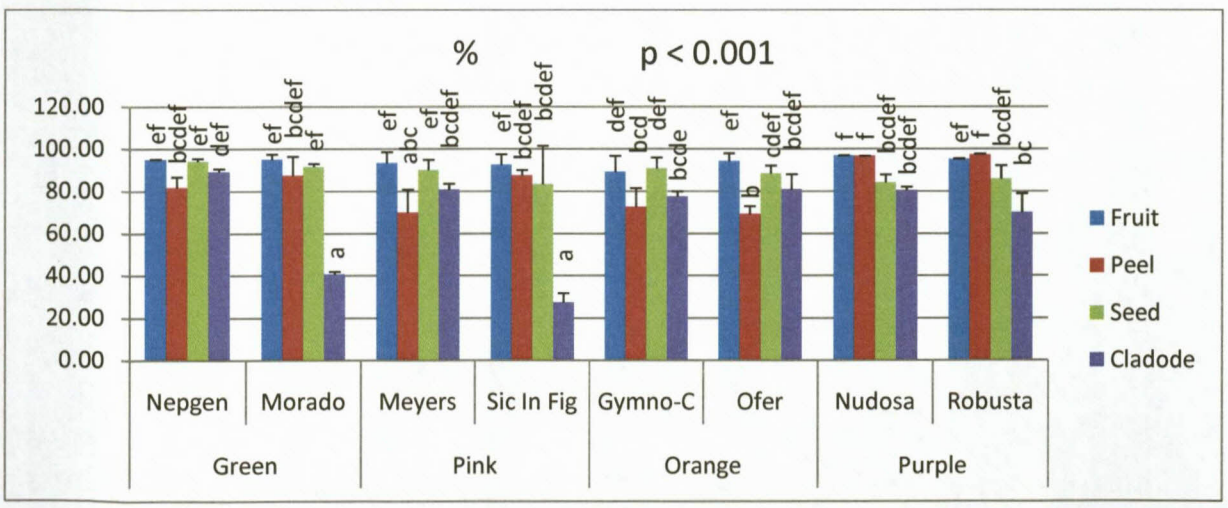


Figure 3.14: The effect of colour, cultivar and tissue type on Chelating activity

3.5 Summary of the combined effect of tissue type, colour and cultivar on antioxidant content.

Cultivar

The cultivar that contained the highest antioxidant content and –potential was *O. robusta* Robusta. Robusta had, by far, the highest Betalain contents, while it had fairly high Carotene and Phenolic levels, but regarding antioxidant capacity, it had the best values for % DPPH and Chelating activity. The second best cultivar would be Gymno-Carpo as it had high Ascorbic acid, Phenolic and Carotene levels and had relatively high antioxidant capacity. Ofer would be the third best cultivar, shadowed by Gymno-Carpo in every aspect; it had high Ascorbic acid, Phenolic and Carotene levels as well as antioxidant capacity.

Colour

If colour should be related to antioxidant levels or -capacity, it appears that the purple fruit Robusta had the highest levels, then orange, then pink and lastly green. There is very little to choose between pink and green cultvars, pink had slightly higher levels of carotene than green, while green had higher phenolic content than pink.

Tissue type

Even though it is clear from the data in this study that antioxidants are abundantly present in the fruit, peel, seeds and cladodes of cactus pear plants, the seeds were (as expected) the tissue type with the highest antioxidant potential. It was explained before though, that it is unclear whether the antioxidants in seeds would be available to the human intestinal track. This suggests the need for further studies on the antioxidant potential of cactus pear seed oils and supplements containing ground cactus seeds as ingredient (such as grape seed is currently available).

The most interesting result regarding tissue type was observed in the antioxidant capacity tests; when % DPPH was tested, peel and cladodes were consistently the highest, while in the % Chelating acitivity tests, fruit and seeds were the best tissue types. It may seem that tissue types have different antioxidant capacity that correlates with their specific function in the plant; peel and cladodes have a protective function while fruit and seeds have a reproductive purpose.

3.6 Conclusion

Antioxidant capacity determination methods employing spectrophotometric absorbance between 400 and 600 nm and may not be accurate in coloured in cactus pear fruit products since Betacyanins and Betaxanthins excert absorbance at 540 and 480 nm respectively. Thus correct readings cannot be taken. It is vital to make use of correct blanks in order to separate the colour readings from the readings of the reagents.

The cultivars that were determined to be the best regarding antioxidant levels and –capacity were *O. robusta* Robusta (purple), *O. ficus-indica* Gymno-Carpo (orange) and *O. ficus-indica* Ofer (Orange) (in descending order). It also seems as if high antioxidant content and -capacity can be associated with pigmentation or colour as purple and orange coloured fruit had the darkest pigmentation and was also determined to be the fruit with the highest antioxidant potential. In antioxidant potential tests, all the tissue types were equal, although fruit and seeds had higher capacities to chelate ferrous ions, peel and seeds showed higher radical scavenging capabilities.

It is clear from this study though, that antioxidants do not act alone, in fact, specific antioxidants cooperate in cactus pear fruit to achieve the exceptional antioxidant potential that they possess, as was suggested by Gülçin (2012). It could thus be concluded in this study that Betalains (Betacyanins and Betaxanthins) group with Carotene in purple fruit (Robusta), while Ascorbic acid, Phenolics and Carotene group in orange fruit (Gymno-Carpo and Ofer). It is believed that the antioxidants that group together also work together synergistically.

Further investigation

From the results of the study on fresh cultivars, five cultivars were chosen with the best overall results for further investigation in processed products. Both the orange fruit cultivars were selected as they demonstrated the highest overall antioxidants. Both will be included in further study.

Gymno Carpo and Ofer had high Ascorbic acid, Carotene and Polyphenol values in the fruit, peel and cladodes.

The pink coloured cultivar that was chosen was Meyers, as it tested high in Ascorbic acid and was overall a better cultivar than Sicilian Indian Fig. Additionally, Meyers demonstrated very

good results overall and was the best quality fruit in earlier studies done in South Africa (de Wit *et al.*, 2011).

Nepgen (green) was selected as it was high in Ascorbic acid in the fruit and demonstrated exceptional results in the Polyphenols in the cladodes.

The purple fruit of *O. robusta* Robusta was included as it is one of the few known sources of betacyanins and contain high levels of betalains in both the fruit and cladodes.

Since most processed products do not include the seeds, it will be omitted for the tissue types studied hereafter.

Subsequently, the antioxidant contents and capacity for processed products will be investigated in order to have complete information available on the antioxidant capacity of *Opuntia-ficus indica* fruit, peel and cladodes.

Chapter 4

Antioxidant content and -potential in processed products from the fruit and cladodes of cactus pears.

4.1 Introduction

Cactus pears (*O. ficus-indica*) originated from Mexico where it is a popular fruit because of its fairly high sugar content and low acidity that gives it a deliciously sweet taste. At the same time these characteristics cause it to be very susceptible to microbial invasion. It is only harvested for a few months of the year and storage time is limited. Food technologists are challenged to develop procedures to lengthen storage life and to diversify by producing different preserved products (Sáenz, 2000; Joubert, 1993; Piga *et al.,* 2003). Several products are obtained from processing the fruit and cladodes. Some of the traditional and known products are juice, marmalades, jellies, jams, dried sheets, pickles, candy and alcoholic drinks. Recently developed products include sweeteners, frozen fruit, cladode flour, oil (from the seeds), mucilage (from cladodes), pigments and dietary fibre (from the cladodes) (Sáenz, 2000). There has been increased interest for products derived from the cactus pear plant due to their potential nutraceutical effects. Compounds found in these products are known to combat oxidative stress and chronic diseases (Chavez-Santoscoy *et al.*, 2009).

Cactus pear fruit is known to be rich in antioxidants such as ascorbic acid, tocopherols and beta-carotenes and it has trace amounts of niacin, riboflavin and thiamine and vitamin K (Feugang *et al.,* 2006; Stintzing & Carle, 2005). More recently the importance of cactus pear fruit as a source of bioactive compounds in relation to other commonly consumed fruit was stressed when high levels of phenols, betalains (Castellanos-Santiago *et al.,* 2008; Figueroa-Cares *et al.,* 2010), taurine (Tesoriere *et al.*, 2005, Fernández-López *et al.,* 2010) and flavonoids such as quercetin, isorhamnetin and kaempferol (Fernández-López *et al.,* 2010) was reported.

Cactus pear juices contain at least twice the antioxidant capacity of strawberry-, plum-, orange-, grapefruit-, red- and white grape-, kiwi-, apple-, pear- and tomato juice (Wang *et al.,* 1996) and

values are in the same range as red wine, pomegranate, concord grape, blueberry and black cherries (Seeram *et al.* 2008). In fact, in the study done by Chavez-Santoscoy *et al.* (2009) the cactus pear cultivar with the lowest tested antioxidant capacity (Amarillo) was compared to red grapes and blueberries that are considered to have high antioxidant capacity. The concept of antioxidant potential in processed foods is gaining momentum and is emerging as an important measure to ascertain the quality of a processed product. In future, the antioxidant capacity measure may take the place of nutrition labelling on products (Herken and Guzel, 2010).

Bacteria, yeast and mould that occur naturally in food causes it to spoil when it grows and increases uncontrollably. It causes chemical and physical changes that render the food inedible or hazardous to eat. In order for the microorganisms to grow, it needs a source of nutrients, moisture, air and favourable temperature and pH. Therefore, in order to prevent the spoilage of food, attention should be paid to control the conditions in and around the food to prevent the microorganisms from flourishing (Bennion, 1985).

There are different options available in the food industry to protect food from harmful microorganisms. Preservatives are chemical substances that will inhibit growth of undesirable microorganisms. Common, household preservatives are salt, sugar, spices, smoke and acidic ingredients such as acetic acid in vinegar are commonly used in the preservation of fruit and vegetables. Sugar in large amounts is used to preserve fruit in the form of jam, preserves and marmalade. Chutney, pickles and atjar are made when using vinegar, salt and spices as preservatives. The reduction of moisture is one of the oldest methods of preservation as dried food contains too little moisture for the microorganisms to increase. Dried fruit has an intermediate moisture content of about 10 to 40 % yet it is pleasant to eat. Preservation by temperature control is effective, as heat destroys microorganisms. Pasteurization involves heating the product to temperatures lower than those required for sterilization; while this heat treatment will not cause all microorganisms to be destroyed, the flavour of the product remains unaffected, thus providing a temporary preservation that prolongs the shelf life (Bennion, 1985, Voedselpreservering, 1986; Van Zyl *et al.,* 1987).

During the processing of fruit juice for long term storage, thermal treatment is used to sterilize the product from bacteria, fungi and to inactivate enzymes (Gurrieri *et al.*, 2000). The industrial processing of fruit in order to obtain juice can affect the antioxidant content as it includes treatments that damage the antioxidants (Tesoriere *et al.*, 2005). Therefore it was assumed that processing will damage antioxidant content and –capacity. Recent studies have disputed this

belief. The instability of ascorbic acid and β-carotene after thermal treatment was confirmed by Tesoriere *et al.* (2005) but the vitamin E and betalains appeared to be preserved. Piga *et al.* (2003) found no significant variations in film packaged fruits during cold storage and described the reason as the ascorbate-sparing effect of polyphenols. There was also no significant decrease in antioxidant capacity values therefore it was concluded that minimal processing did not decrease the ascorbic acid and polyphenol content or capacity. Salvia-Trujillo *et al.* (2011) found that 97% of ascorbic acid was retained in fruit juice and milk beverages after high-intensity pulsed electric fields (HIPEF) thermal processing.

When Jaramillo-Flores *et al.* (2003) investigated the cladodes for carotene content after thermal treatment; it was found that the carotenoid extractability increased with thermal treatments. Rickman *et al.* (2007) confirmed that processing methods such as canning impoved the extraction of carotene from its cell matrix, causing higher carotene levels in thermally processed products. This effect might be as a result of complexes formed with mucilage because of the high degree of pectin esterification that cannot be detected at pre-gelling temperatures. In fact, the higher the temperature treatment, in the study by Jaramillo-Flores *et al.* (2003), the more carotene was released from the cell matrix because of the thermal denaturation of carotenoid-protein, tissue softening and higher penetration of organic solvents into the cells. Medina-Torres *et al.* (2011) found that the bioactive compounds are preserved in cladodes during the dehydration process as 25% of Flavonoids, 20% of Ascorbic acid and 50% Carotene remained intact. Stintzing *et al.* (2005) demonstrated that Betalains keep their appearance over a large pH spectrum from pH 3 to 7.

Ryan and Prescott (2010) did not only investigate the antioxidant activity of different commercial juices before digestion but also after digestion. In doing so, very interesting results were seen and this uncovered a whole new issue on whether heat treatments do, in fact, lower antioxidant capacity in processed fruit products. In some juices studied (such as pomegranate juice), the fresh juice displayed higher antioxidant capacity than the long life juice. But other juices such as orange, grapefruit and red grape had higher antioxidant capacity in long life versions. This should not happen according to traditional beliefs that individual antioxidants are destroyed by heat and therefore its ability to scavenge. It was investigated by Ryan and Prescott (2010) and found that many other authors have reported the same phenomenon and possible explanations were reported as follows:

- Antioxidant capacity increased because the heat disrupts the cell walls allowing more antioxidant components to be released;
- Heat treatments deactivate oxidative enzymes that would normally destroy antioxidants;
- Heat treatments cause the formation of new structural groups which enhance antioxidant activity.

The final explanation was seen by Ryan and Prescott (2010) as the most correct one; as it was found before that antioxidant capacity of gallic acid increased after heat treatment because of the formation of new hydroxyl groups and that structure changes in polyphenols increases antioxidant activity. If structural changes do occur in antioxidants during heat exposure, it could not be detected while, in fact, the antioxidant capacity of the product did not decrease (as seen in this study). Therefore it was theorized (Ryan & Prescott, 2010) that heat treatment can possibly increase antioxidant potential by causing slight changes that take place in the structure of the compounds. These changes render the antioxidant more stable to pH, allowing it to continue its activity throughout the digestive tract.

This part of the study investigated the betalain-, ascorbic acid-, carotene- and total phenolic contents in preserved products to compare the antioxidant levels of fresh (as discussed in Chapter 3) and preserved products. Only the five cultivars with the highest antioxidant content from the previous part of the study (Chapter 3) have been chosen for this purpose. From the green cultivars Nepgen was chosen, from the pink cultivars it was Meyers and from purple cultivars, Robusta was chosen (Robusta being a different species namely *O. robusta*). For the orange cultivars, both cultivars gave outstanding results and both Gymno-Carpo and Ofer were thus included in the study. Different preservation methods were applied to each of the five cultivars on the pulp, peel, seeds and cladodes in order to obtain results for antioxidant content and potential in each part of the plant after preservation methods were applied.

It was important to produce marketable products for the South African public. The products had to be well known, everyday food that South Africans are accustomed to. Most products are usually without seeds, therefore seeds were omitted in the preservation process and recipes were chosen that would suit the type of fruit (sweet, nonacidic and soft) as well as the cladodes (tough vegetable). Juice was the first choice as it would be the most obvious product to market since the fruit has such vibrant colours. Drying is the oldest and one of the easiest preservation methods and therefore had to be included for study. Chutney was included as this method includes sugar, acid as well as spices in the preservation technique. Preserved whole fruit in

syrup was included since it presents very well and delivers a very tasty product. Pickling was only done on the cladodes as it is a technique that is only done on acidic type fruit and the cladodes has the flavour as well as the turbidity for successful pickles (Voedselpreservering, 1986).

4.2 Materials and methods

4.2.1 Sample collection and preparation

Fruit was collected from an experimental orchard outside Bloemfontein (as discussed in Chapter 3.2.1) at 50% colour-break stage to ensure an even degree of ripeness. Fruit from the five chosen varieties were picked and transported to the laboratory. It was immediately refrigerated at 4°C and processed within three days. The fruit can be stored for six weeks at the correct temperature (10 °C) and humidity (90%) (Joubert, 1993) and as it is classified as a non-climacteric fruit, it does not ripen or change after harvesting.

Working with cactus pear fruit is problematic as it is covered with hair-like thorns that penetrates the skin and causes severe discomfort. Therefore the first challenge was preparing the fruit by removing the thorns. For this purpose the fruit was washed in cold running water. It was brushed individually by holding each fruit with tongs and then placed in boiling water for thirty seconds and immediately shocked in cold water. The washing and blanching of the fruit removed enough thorns to be able to work with the fruit comfortably without gloves and the outer skin of the peel could be easily pulled free of the peel similar to the way the outer skin of a tomato is removed after blanching (Brown, 2008). After removing the skin, the peel was separated from the fruit by cutting the fruit from end to end and pulling the peel away to reveal the inner fruit (cut and tear method).

4.2.2 Processing

4.2.2.1 Preparation of the cactus pear fruit and cladode juices

Fruit juice was prepared in triplicate by using three individual fruit according to the method by Gurrieri *et al.*, 2000). After the fruit was washed and rubbed clean of glochids, it was weighed and the skin and peel removed as described in 4.2.1. Both the peel and fruit were weighed separately, liquidized using a Milex 4-in-1 multi-purpose Mean Juice Machine (model MMJ004) and strained (0.5 mm mesh size) to remove all the seeds. Cladode juice was prepared by peeling three cladodes, liquidizing the inner soft part and strained (0.5 mm mesh size) to remove the tough parts. No water was added to the fruit or cladode juice. The filtered juice was pasteurized in a water-bath for ten minutes until the internal temperature reached 72°C and

immediately shocked in cold water. It was then frozen in aliquots until further analysis (not longer than two months). The bright colours of the samples from the different coloured fruit cultivars are seen in Figure 4.1.

Figure 4.1: Juice made from four differently coloured cactus pear fruit

4.2.2.2 Drying of the cactus fruit and cladodes

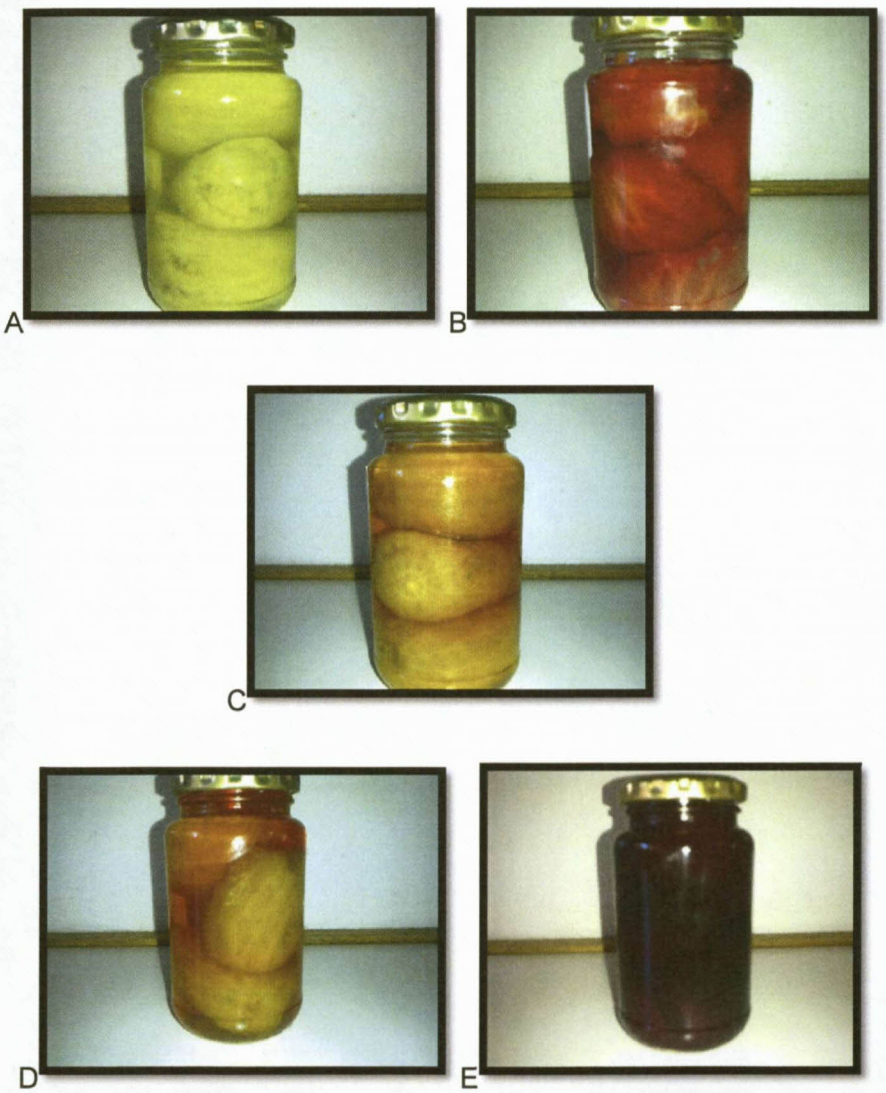
Three individual fruit and cladodes were cut, washed and blanched at 80°C for 5 minutes. The fruit was cut into slices and the cladodes were cut into thin lengths. It was dried in a convection oven set at 90°C for 18 hours (Voedselpreservering, 1986). It was then vacuum packed and frozen until further analysis as seen in Figure 4.2.

Figure 4.2: An example of the prepared dried fruit products

4.2.2.3 Canning of fruit and cladodes (whole preserves)

In order to preserve the fruit whole, each fruit was washed and blanched and by using a very sharp knife the two ends were cut away. The knife was wedged between the thin outer skin of the peel (that resembles the skin of a tomato) and the flesh part of the peel and run around to remove the inedible outer skin from the edible inner part of the peel (Voedselpreservering, 1986).

Fruit was preserved in triplicate according to the open-kettle method (Van Zyl *et al.*, 1986). The fruit was cooked in boiling water for five minutes until just tender. It was transferred into prepared boiling syrup (250 ml sugar and 500 ml water) for ten minutes to allow the syrup to permeate through the fruit. All utensils, jars and lids were sterilized in boiling water. The preserved fruit was transferred to hot, sterilized jars. The jars (as seen in Figure 4.3) were filled with boiling syrup to the brim in order to exclude air from the jar. Metal lids were used to cover the jars and screwed down tightly. The steam from the fruit condensed and formed a vacuum that completed the seal. It was cooled down, labelled and stored at room temperature (Voedselpreservering, 1986).



A: Nepgen; B: Meyers; C: Ofer; D: Gymno-Carpo; E: Robusta

Figure 4.3: *Whole preserved fruit made from each of the five cultivars.*

Traditionally, vegetables are not preserved in syrup the same way as fruit is, but rather in brine (Voedselpreservering, 1986). Cladodes were therefore preserved in brine (in triplicate) as seen in Figure 4.4 according to the steam pressure method (Van Zyl *et al.,* 1986). The cladodes were washed and cut into thin strips of approximately 20 x 150 mm. Strips were dipped in boiling water to prevent them from shrinking during the preservation process. The strips were neatly packed into sterilized, hot jars, with a capacity of 500 g and filled with hot brine solution consisting of 2.5 g salt, 3 g vinegar and 2 g sugar and 500 ml water. A space of 5 mm was left at the top of the jar to allow for the expansion of the cladode strips and the bubbling of the liquid. The lid was placed on and turned back by half a turn. A pressure cooker was filled with hot water to a depth a 50 mm - 70 mm and the false bottom placed in position. The jars were placed on the false bottom, taking care that they did not touch. The pressure cooker was sealed and the pressure allowed to gradually rise. After it was kept at pressure for 25 minutes, it was removed from the heat source and allowed to cool down gradually. The jars were lifted out with tongs, the lid screwed on tightly, labelled and stored at room temperature (Bennion, 1985).

Figure 4.4: An example of the preserved cladodes made from each of the five cultivars

4.2.2.4 Fruit- and cladode chutney

In order to make chutney from the peel and pulp separately, 20 cactus fruit were prepared by removing the outer skin (as explained in 4.2.2.3) and the peel and fruit separated. The peel and pulp were liquidized separately and the seeds filtered out. The total weight of fruit and peel was weighed and the ingredients determined as indicated in Table 4.1 (Voedselpreservering, 1986).

Table 4.1: The chutney formulation for fruit and peel

Ingredients	%
Fruit (pulp or peel)	69
Sugar	13.8
Cayenne pepper	0.0138
Onion, minced	3.45
Salt	0.22
Ginger, powdered	0.22
Mustard, powdered	0.06
Garlic, powdered	0.24
Vinegar, white, grape	12.94
Total	**100**

(Voedselpreservering, 1986)

The radiant colours of fruit chutneys that the different cactus pear cultivars presented are seen in Figure 4.5.

Figure 4.5: The fruit (pulp) chutneys made from each of the five cultivars

Cladodes were washed and the pulp was obtained by feeding cladodes through a juicer (Milex 4-in-1 multi-purpose Mean Juice Machine, model MMJ004). The juicer removed the peel and

fibres and liquidized it into pulp. The total weight of cladode pulp was obtained and the chutney recipe was determined as follows (Table 4.2) (Voedselpreservering, 1986). Chutneys made from cladodes did not have an attractive colour (Figure 4.6).

Table 4.2: The chutney formulation for cladodes

Ingredients	%
Cladode pulp	65
Sugar	10
Cayenne pepper	0.01
Onion, minced	4
Salt	0.2
Ginger, powdered	0.2
Mustard, powdered	0.06
Garlic, powdered	0.2
Vinegar, white, grape	10
Total	**100**

(Voedselpreservering, 1986).

Figure 4.6: A cladode chutney

All the ingredients were added to a stainless steel saucepan and allowed to boil slowly until it was thick and dropped off the spoon in flakes and had the consistency of jam. It was stirred occasionally using a wooden spoon. The prepared chutney was poured into three hot sterilized jars and sealed immediately, labelled and stored at room temperature (Van Zyl *et al.*, 1986).

4.2.2.5 Pickling of cladodes

The fruit of the cactus pear plant is not suitable for pickling as it is too sweet. Only sour tasting fruit such as sourapples, quinces and plums are pickled with success. Cladodes are however suitable as it has a slightly sour taste that could be successfully pickled (Van Zyl *et al.,* 1986). Pickled cladodes may be an acceptable product to the South African community, for it is not cooked (turbidity and colour stays intact), and the flavour is enhanced by the spicy vinegar. Cladodes were weighed, washed and cut into thin trips of approximately 20 by 150 mm. It was left for 20 hours in brine. The brine, consisting of 500 g salt and 5 liters of water, was previously boiled, strained and allowed to cool. After removing the cladodes from the brine they were thoroughly rinsed in cold water and packed into three jars up to 20 mm from the rim. The jars were filled with prepared spicy vinegar up to at least 10 mm above the contents to exclude air and avoid discolouration. Jars were sealed and allowed to mature for two months at room temperature. The spicy vinegar consisted of 2 l of vinegar, 9 g mustard seeds, 5 g allspice, 2 g whole cloves and 0.5 g foil. It was prepared by boiling the ingredients together briefly and allowing it to steep for two hours (Voedselpreservering, 1986). Pickles that were made from each of the five cultivars are seen in Figure 4. 7.

Figure 4.7: Pickles made from the cladodes of each of the five cultivars

4.2.3 Sample preparation for antioxidant content analysis

Representative samples were taken from each of the preservation processes (in triplicate). Regarding the carotene content, separate pulp, peel, and cladode samples from each product were frozen at -18°C until further analysis was done using a hexane extract of the product (section 3.2.2). In preparation for the analysis of the other antioxidants (ascorbic acid, betalains and phenolic compounds) aqueous samples of the pulp, peel, seeds and cladodes were prepared. The added amounts of distilled water were recorded in order to recalculate the data correctly. Regarding the fruit products, the peel was removed and liquidized with added water.

The pulp was liquidized with added water and the seeds were strained out. The cladodes were liquidized and water added. All samples were homogenized using an ultra-turrax T25 for 30 seconds and centrifuged using a Beckman at 8000 rpm for 10 minutes at 4°C. The supernatant was frozen in aliquots as described above (section 3.2.2).

4.2.4 Antioxidant content and potential determinations

Antioxidant content and potential determinations were done on the extracts (explained in 4.2.3.) according to the methods discussed in Chapter 3. All determinations and data were done in triplicate and averages obtained subsequently.

4.2.5 Statistical analysis

Statistical analysis was done as discussed in Chapter 3. These include analysis of variance (ANOVA), analysis of the mean values to determine the effect of cultivar and product type and colour on the antioxidant properties and a Pearson correlation analysis was done to determine the correlation between antioxidant content and capacity with individual products and cultivars. Principal component analyses (PCA) were done on products and cultivars to determine the associations between antioxidants and antioxidant potential.

4.3 Results and discussion

4.3.1 The effect of cultivar and product type on the antioxidant properties of fresh and processed cactus pear tissue types

To determine the effect of cultivar and product type on the antioxidant properties of fresh and processed cactus pear tissue, the fruit, peel and cladodes will be investigated separately in Tables 4.3, 4.4 and 4.5.

4.3.1.1 Fruit

The mean values for the effects of cultivar and product type on the different antioxidants for fresh and processed fruit (pulp) are indicated in Table 4.3.

According to Sacchetti *et al.* (2008), the antioxidant potential of processed fruit products after processing and storage is mostly dependant on the quality of the fresh fruit, the type of process and the storage conditions. It is therefore important to investigate the different cultivars that were used in each product type in order to find the best quality cultivar coupled with the best processing techniques.

Regarding the antioxidant potential results, the Chelating activity in fresh fruit (89.17 – 95.09%) and the processed juice (85.83 – 96.49%) was generally higher than that of the chutney (85.17 - 93.99%), dried fruit (85.00 – 95.38%). Preserves (70.00 – 90.50%) had the lowest levels. In terms of cultivar, most cultivars had similar (statistically significant) results. It could be derived from the data that overall, Robusta (90.50 – 96.49%) was the best cultivar and Meyers (70.00 – 95.38%) had the lowest results. Robusta juice would deliver the best product in terms of % Chelating activity. Sumaya-Martinez *et al.* (2011) found lower values of 51% to 74%, when they investigated purple, red, yellow and white cactus pears. In their study the highest and lowest values were from fruit of different purple cultivars. It was concluded in that study that Chelating activity does not depend on the colour of the cactus pear. In this study it appears that purple fruit will have the highest Chelating ability.

Percentage DPPH tests revealed (Table 4.3) results that were quite similar within products; in other words, there were very few significant differences between cultivars used for the same product. All the products and cultivars had excellent radical scavenging ability (66.99 – 98.98%) and differences that exist amongst products are small. Juice (93.53 – 96.10%), chutney (92.02 – 97.42%) and dried products (92.05 – 98.98%) had statistically similar results while the results for fresh fruit (70.12 – 83.63%) and preserved products (66.99 – 93.56%) were slightly but significantly lower. Juice was the best product overall by very slight margins and fresh fruit had

the lowest radical scavenging ability. Nepgen, together with Robusta, were the cultivars with slightly higher % DPPH readings. This is an interesting result as Robusta had exceptionally high Betalain values but Nepgen did not have the highest antioxidant content of any individual antioxidant determination tests. Gymno-Carpo and Ofer (two orange cultivars) had the lowest readings by very slight margins.

The ability to scavenge radicals (% DPPH) in cactus pear fruit juice according to the current study (85.83 – 96.49%) was higher than leading Ready-to-Drink Polyphenol-Rich Beverages from USA tested by Seeram *et al.* (2008), who reported values in pomegranate juice (50.1%), concord grape juice (28.2%), black cherry (11.3%), cranberry (19.2%), orange juice (12.7%) and iced green teas (22.3%). The values in this study were more similar to Tesco and Ocean Spray commercial fresh and long life juices, such as red grape (79.5%), cranberry (83.8%) and pomegranate juices (84.8%) juices, tested by Ryan and Prescott (2010). Borges *et al.* (2010) found a great variability in antioxidant potential between pure and blended fruit juices, but results showed that juices rich in phenolics had the highest antioxidant capacity. Ascorbic acid contributed greatly to antioxidant capacity in juices that were supplemented with Vitamin C after pasteurisation.

In both antioxidant capacity tests (% DPPH and % Chelating ability) the preserves had the lowest capacity. It may seem that juice and chutney will give the best combined results as far as cactus pear fruit is concerned; in fact, Robusta is the cultivar that yielded the best results overall.

Dried fruit (83.21 -154.08 mg/100 g) yielded the highest Ascorbic acid values, while preserves (17.02 – 32.48 mg/100 g) had the lowest values in all cultivars. This could be explained by the concentrated nature of dried fruit together with the low temperature preservation techniques applied, in contrast to the high temperature applied to preserved fruit. Gymno-Carpo (orange) was the cultivar with the highest results (except for juice) and Robusta (purple) was the lowest (statistically significant) overall in products. Gurrieri *et al.* (2000) detected a 50% loss of Vitamin C in processed juices of cactus pear fruit and Tesoriere *et al.* (2005) found that the Vitamin C content reduced from 1.75 to 0.17 mM after pasteurization and concentration. In the current study the loss from fresh to processed juice was 54.7% on average but the loss differed among the different cultivars. In Meyers, the loss was only 8% while in Nepgen it was 70%. Meyers had the highest Ascorbic acid values in fruit-, peel (Table 4.2) - and cladode (Table 4.3) juices. It was suggested by Borges *et al.* (2010) that Vitamin C is added to several fruit juices after

pasteurization and that this supplemented vitamin C, rather than the naturally occurring constituents, boost the antioxidant capacity of processed juices.

The Betacyanin and Betaxanthin combined content results indicated that all products and cultivars had statistically similar (low) results (0.44 – 14.83 mg/kg) except for Robusta that had significantly higher readings in fresh (126.60 mg/kg) and dried (176.08 mg/kg) products. In the fresh Robusta fruit the Betacyanins are high (74.47 mg/kg) however in the fruit juice it goes down to 5.46 mg/kg. This significant drop should be investigated in subsequent studies and could be explained if the Betacyanins are part of the soluble solids which are removed during purification of the juice or by the heat processes of juice making. It is clear that fresh and dried Robusta would be the best product for Betalain contents but it is noteworthy that all chutney products had promising results (better than fresh) for Betalains notwithstanding the processing method applied.

As carotenoids are lipid soluble, it was not significantly lost in water during processing and storage. In fact, carotene may have a greater ability to scavenge radicals after processing, such as was reported in tomatoes (Rickman *et al.,* 2007). Canning and other heat treatments improved extraction and higher carotene levels were found in processed products, but excessive heat may lead to its degradation (Rickman *et al.,* 2007). Carotene concentrations in canned carrots were retained in another study by Howard *et al.* (1999) during prolonged storage periods and microwave cooking. Carotene content was the lowest in preserves (0.27 – 1.76 mg/kg) but it remained low (statistically similar) in all products except for the significantly higher values found in Gymno-Carpo (21.87 µg/g), Robusta (17.14 µg/g) and the highest value in Ofer (39.6 µg/g) dried products. The lowest Carotene levels were observed in all Nepgen (green) products (average 0.91 µg/g). The values found in cactus pear dried fruit were higher than values reported in prunes (1.6 mg/100 g dw), apricots (10.7 mg/100 g dw), raisins (2.2 mg/100 g dw) and figs (11.0 mg/100 g dw) (Ouchemoukh *et al.,* 2012). It was found in this study (3.4.7.3) that orange and purple fruit contained the highest levels of carotene, similar to what was found in these results in processed products.

Phenolic content increased in processed products. It is uncertain if this increase could be explained by the same phenomenon that occurred in carotene, where heat treatments released the antioxidants from the mucilaginous substances in cactus pear tissue. The lowest readings were found in fresh fruit with Ofer the lowest (13.41 mg/kg) and the highest content in dried fruit of Robusta (326.33 mg/kg). These phenolics concentrations correlated well with values found in

dried fruit from Algeria, namely prunes (0.77 g/100 g GAE dw), apricots (0.65 g/100 g GAE dw), raisins (0.65 g/100 g GAE dw) and figs (0.47 g/100 g GAE dw) (Ouchemoukh *et al.,* 2012).

Interestingly, the Phenolics content in Chutney (98.89 – 150 mg/kg) was slightly lower than dried fruit but was surprisingly high, indicating that chutney may contain high Phenolic levels. In terms of cultivar, there was not one single cultivar that contained significantly more or significantly less Phenolics than other cultivars. This data reflected the same as findings in Chapter 3 (Figure 3.12), where it was concluded that the colour and cultivar do not seem to have an effect on Phenolic content. Meyers had the highest levels of Phenolics in the fruit juice (96.33 mg/kg). In a study done on apples (Sacchetti *et al.*, 2008), antioxidant capacity was correlated with phenolics. When antioxidant capacity increased during storage of dried and pureed apples, the explanation was given that enzymatic reactions other than oxidation (hydrolysis of flavonoid glycosides) took place during the drying process, causing novel phenolic modification that made phenolics more available.

It could be derived from the data in Table 4.3 that, as far as processed products manufactured from the fruit of the cactus pear is concerned, the best product was dried fruit and secondly chutney, with juice in third position. Preserves had the lowest antioxidant content and -capacity. The antioxidants survive nonetheless and could contribute to antioxidant levels in the human body. Vinson *et al.* (2005) recommended dried fruit as a snack, as it has a greater nutrient density and is a good choice for a high antioxidant food, while Herken and Guzel (2010) recommended fruit juices as it is more digestible than other products and good souces of antioxidants.

Table 4.3: The effect of cultivar and product type on the antioxidant properties of fresh and processed cactus pear fruit.

Product	Cultivar	Chelating activity %	DPPH %	Ascorbic acid mg/100g	Betacyanins mg/Kg	Betaxanthins mg/kg	Betacyanins + Betaxanthins mg/kg	Carotene ug/g	Phenolics mg/kg
Fresh	Gymno C	89.17 ± 7.64^{abcd}	70.12 ± 4.99^{bc}	95.27 ± 21.00^{def}	1.54 ± 0.28^{a}	1.08 ± 0.20^{a}	2.62 ± 0.48^{a}	1.86 ± 0.09^{a}	22.08 ± 11.81^{ab}
	Meyers	93.33 ± 5.20^{bcd}	58.65 ± 11.49^{a}	52.52 ± 5.75^{abcde}	2.71 ± 0.15^{a}	1.90 ± 0.10^{a}	4.62 ± 0.25^{a}	0.92 ± 0.16^{a}	17.45 ± 9.15^{a}
	Nepgen	94.94 ± 0.37^{cd}	83.63 ± 1.30^{def}	91.69 ± 8.98^{def}	1.83 ± 0.55^{a}	1.28 ± 0.39^{a}	3.11 ± 0.94^{a}	0.62 ± 0.19^{a}	16.72 ± 7.48^{a}
	Ofer	94.17 ± 3.82^{cd}	77.56 ± 4.61^{cde}	94.07 ± 29.97^{def}	1.20 ± 0.17^{a}	0.84 ± 0.12^{a}	2.05 ± 0.28^{a}	1.69 ± 0.32^{a}	13.41 ± 1.62^{a}
	Robusta	95.09 ± 0.38^{cd}	77.05 ± 4.53^{bcde}	32.38 ± 22.99^{abc}	74.47 ± 41.06^{b}	52.13 ± 28.74^{b}	126.60 ± 69.81^{b}	1.58 ± 0.09^{a}	18.47 ± 6.02^{a}
Chutney	Gymno C	89.17 ± 1.44^{abcd}	94.89 ± 1.64^{gh}	74.94 ± 15.66^{bcdef}	8.07 ± 2.54^{a}	5.65 ± 1.77^{a}	13.72 ± 4.31^{a}	0.79 ± 0.30^{a}	98.89 ± 2.22^{abcde}
	Meyers	76.67 ± 1.44^{abc}	92.06 ± 0.17^{fgh}	42.49 ± 12.72^{abcd}	4.89 ± 0.43^{a}	3.42 ± 0.30^{a}	8.31 ± 0.73^{a}	0.78 ± 0.19^{a}	143.27 ± 4.72^{bcde}
	Nepgen	89.17 ± 3.82^{abcd}	92.02 ± 0.08^{fgh}	38.62 ± 3.37^{abcd}	8.72 ± 3.52^{a}	6.11 ± 2.47^{a}	14.83 ± 5.99^{a}	1.36 ± 0.37^{a}	114.14 ± 22.40^{abcde}
	Ofer	85.00 ± 2.50^{abcd}	97.42 ± 1.24^{h}	49.54 ± 9.96^{abcde}	3.85 ± 0.60^{a}	2.69 ± 0.42^{a}	6.54 ± 1.02^{a}	2.34 ± 3.15^{a}	137.04 ± 4.42^{abcde}
	Robusta	93.99 ± 0.69^{cd}	95.57 ± 0.10^{gh}	15.03 ± 0.53^{a}	31.25 ± 2.78^{a}	21.88 ± 1.95^{a}	53.13 ± 4.73^{a}	0.34 ± 0.11^{a}	150.83 ± 6.11^{cde}
Dried	Gymno C	85.00 ± 15.61^{abcd}	92.56 ± 0.14^{fgh}	154.08 ± 20.80^{g}	3.58 ± 1.43^{a}	2.50 ± 1.00^{a}	6.08 ± 2.43^{a}	21.87 ± 3.59^{b}	184.31 ± 31.34^{e}
	Meyers	95.38 ± 0.80^{cd}	92.05 ± 0.30^{fgh}	106.30 ± 40.74^{efg}	5.92 ± 3.54^{a}	4.14 ± 2.48^{a}	10.06 ± 6.01^{a}	4.48 ± 0.65^{a}	175.10 ± 55.12^{cde}
	Nepgen	74.17 ± 15.07^{ab}	98.98 ± 0.11^{h}	83.21 ± 19.27^{cdef}	1.84 ± 0.26^{a}	1.29 ± 0.18^{a}	3.13 ± 0.44^{a}	2.15 ± 0.27^{a}	121.20 ± 109.32^{abcde}
	Ofer	89.17 ± 1.44^{abcd}	92.11 ± 0.03^{fgh}	130.40 ± 31.43^{fg}	2.53 ± 0.71^{a}	1.77 ± 0.49^{a}	4.31 ± 1.20^{a}	39.60 ± 7.48^{c}	182.39 ± 54.51^{de}
	Robusta	94.34 ± 1.04^{cd}	96.51 ± 0.09^{gh}	113.59 ± 45.53^{fg}	103.58 ± 35.57^{b}	72.51 ± 24.90^{b}	176.08 ± 60.48^{b}	17.14 ± 1.60^{b}	326.33 ± 131.47^{f}
Juice	Gymno C	95.00 ± 0.01^{cd}	93.91 ± 2.14^{fgh}	38.06 ± 5.08^{abcd}	0.58 ± 0.12^{a}	0.41 ± 0.08^{a}	0.99 ± 0.20^{a}	0.98 ± 0.18^{a}	73.80 ± 13.11^{abcde}
	Meyers	85.83 ± 10.10^{abcd}	96.10 ± 0.57^{gh}	48.48 ± 9.62^{abcde}	1.08 ± 0.28^{a}	0.76 ± 0.20^{a}	1.84 ± 0.48^{a}	0.74 ± 0.07^{a}	96.33 ± 19.81^{abcde}
	Nepgen	95.00 ± 0.01^{cd}	93.53 ± 0.12^{fgh}	28.17 ± 6.09^{abc}	0.49 ± 0.07^{a}	0.34 ± 0.05^{a}	0.83 ± 0.12^{a}	0.31 ± 0.19^{a}	77.94 ± 7.85^{abcde}
	Ofer	91.67 ± 10.10^{bcd}	95.31 ± 1.41^{gh}	44.42 ± 5.39^{abcd}	0.69 ± 0.14^{a}	0.48 ± 0.10^{a}	1.17 ± 0.24^{a}	0.63 ± 0.13^{a}	74.02 ± 13.28^{abcde}
	Robusta	96.49 ± 0.33^{d}	94.69 ± 1.45^{gh}	21.21 ± 5.20^{ab}	5.46 ± 1.39^{a}	3.82 ± 0.98^{a}	9.27 ± 2.37^{a}	2.01 ± 0.44^{a}	58.97 ± 19.79^{abcd}
Preserves	Gymno C	70.83 ± 7.64^{a}	74.16 ± 2.24^{bcd}	32.48 ± 7.92^{abc}	0.57 ± 0.13^{a}	0.40 ± 0.09^{a}	0.97 ± 0.23^{a}	0.55 ± 0.05^{a}	74.32 ± 8.30^{abcde}
	Meyers	70.00 ± 2.50^{a}	90.29 ± 1.84^{fgh}	27.44 ± 10.94^{abc}	0.96 ± 0.08^{a}	0.67 ± 0.06^{a}	1.63 ± 0.13^{a}	0.93 ± 0.08^{a}	86.75 ± 10.73^{abcde}
	Nepgen	78.33 ± 5.77^{abcd}	86.89 ± 7.10^{efg}	22.43 ± 5.76^{ab}	0.28 ± 0.11^{a}	0.19 ± 0.08^{a}	0.47 ± 0.18^{a}	0.27 ± 0.02^{a}	54.50 ± 16.75^{abc}
	Ofer	70.83 ± 8.04^{a}	66.99 ± 1.08^{ab}	22.48 ± 8.53^{ab}	0.26 ± 0.06^{a}	0.18 ± 0.04^{a}	0.44 ± 0.10^{a}	0.68 ± 0.04^{a}	58.38 ± 13.24^{abcd}
	Robusta	90.50 ± 0.35^{bcd}	93.56 ± 0.22^{fgh}	17.02 ± 5.53^{ab}	9.99 ± 1.29^{a}	6.99 ± 0.91^{a}	16.99 ± 2.20^{a}	1.76 ± 0.08^{a}	68.04 ± 3.74^{abcde}
AVG		87.33	87.86	59.05	11.05	7.74	18.79	4.26	97.79
STD		10.01	11.00	41.39	25.93	18.15	44.07	9.00	77.01
CV		11.47	12.52	70.08	234.56	234.56	234.56	211.42	78.75
Significance level		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

Means with different superscripts in the same column differ significantly

4.3.1.2 Peel

To explain the effect of cultivar and product type on the antioxidant properties of fresh and processed cactus peels, the mean values for the effects of cultivar and product type on the different antioxidants for fresh and processed fruit (pulp) are indicated in Table 4.4.

There is very little literature available on fresh cactus pear peels, furthermore no research articles could be found on processed cactus pear peel or processed peels of any other related fruit.

The % Chelating activity for peels was lower than in fruit, with the highest in dried products (74.17 – 96.46%) and the lowest (53.33 – 91.40%) in preserves. Robusta peel was the cultivar with the highest (91.40 – 97.32%) levels throughout all the products, while Meyers had the lowest (53.33 – 95.20%) levels.

In % DPPH tests, chutneys had higher values (94.55 – 97.34%) than dried (93.33 – 95.63%) and fresh (91.18 – 96.25%) cactus peels. The values for fresh, dried and chutney did not differ significantly, but juice and preserves were significantly lower, with preserves having the lowest values. Robusta had the highest values amongst cultivars and Nepgen the lowest, although by very slight margins. Percentage DPPH levels for Nepgen, Meyers, Ofer and Gymno-Carpo peel were lower than in fruit and cladodes. Thus, Robusta had the highest % DPPH levels in peel.

Ascorbic acid levels in peel were lower than in fruit. In peel it ranged from 11.67 mg/100 g in Robusta peel preserves to 109.46 mg/100 g for dried Robusta peels. Therefore, Robusta demonstrated the highest and lowest values for Ascorbic acid. Yet, most products and cultivars had statistically similar results except for the before -mentioned values that were significantly low and high, respectively. The average Ascorbic acid content was 46.71 mg/100 g. The two best products were fresh and dried peels, while chutney and juice had lower (and very similar) results. Preserves had the lowest Ascorbic acid content. Robusta had the highest average (51 mg/100 g) Ascorbic acid content, while Nepgen had the lowest average (40 mg/100 g). The levels for peel juice were approximately 50% that of the values for fresh peel, the same as was found in fruit (discussed in 4.3.2.1) and by Gurrieri *et al.* (2000).

Generally, the values for Betalains (Betacyanins + Betaxanthins) were generally lower in peel than in fruit, but similar in the sense that values for all cultivars were significantly equal (between 0.44 – 11.69 mg/kg), except for Robusta fresh and dried that had values of 72.46 and 72.20 mg/kg respectively. These values in Robusta peels were roughly half that of the values in fruit.

It has been found in the literature that processed products often contain similar amounts of carotene than the fresh counterparts (Rickman *et al.,* 2007). Carotene levels in peels were generally slightly higher than in fruit (Table 4.3) (as was found in Chapter 3), however the levels in dried peels were significantly (approximately 80 times) higher. These high levels could be as a result of the concentrated nature of the dried peel. Robusta was the cultivar with the highest values for all products and Gymno-Carpo would be the second highest cultivar with very little difference between the remaining three cultivars.

Phenolics were higher in processed peel products than fresh peel, as was the case in fruit products. Chutney and dried products had the highest contents of Phenolics and the highest values were recorded for dried Meyers peel (100.96 mg/kg) and Robusta (126.82 mg/kg) peel. Juice had very similar results in all cultivars (47.74 – 59.56 mg/kg), while the values in preserves were not consistent (Table 4.4). Overall though, there were very high individual results but no one cultivar could be singled out as having the highest phenolic content across the different peel products.

It could be concluded that the processed cactus pear peel contains high levels of antioxidant as well as demonstrates high antioxidant capacity. In comparison to fruit, peel had higher % DPPH, Carotene and Phenolics values, while it had lower Ascorbic acid, Betalain and % Chelating activity levels. Therefore, it should be included, where possible, in the products made from the fruit. Alternatively, peels that are normally discarded as waste when only fruit is used, should be utilized for preservation purposes. The best process for peels was drying while the best cultivar was Robusta (as was concluded in section 4.3.1.1). The preserves had the lowest values in terms of antioxidants.

The overall problem that exists with peels and the reason why it is more often than not discarded as waste is that the skin is covered with hair-like thorns (glochids). However, by brushing and blanching the whole fruit, most of the thorns could be removed (as was explained in section 4.2.1) and peels could be used separately.

Table 4.4: The effect of cultivar and product type on the antioxidant properties of fresh and processed cactus pear peels

Product	Cultivar	Chelating activity %	DPPH %	Ascorbic acid mg/100g	Betacyanins mg/Kg	Betaxanthins mg/kg	Betacyanins + Betaxanthins mg/kg	Carotene ug/g	Phenolics mg/kg
Fresh	Gymno C	72.50 ± 9.01^{abcde}	91.18 ± 2.11^{efgh}	68.04 ± 38.66^{bcde}	2.21 ± 0.21^{ab}	1.55 ± 0.15^{ab}	3.75 ± 0.37^{ab}	3.99 ± 1.32^{ab}	14.04 ± 2.48^{ab}
	Meyers	70.00 ± 10.90^{abcd}	96.25 ± 2.91^{gh}	86.28 ± 21.90^{de}	6.87 ± 0.75^{ab}	4.81 ± 0.53^{ab}	11.69 ± 1.28^{ab}	1.79 ± 0.35^{ab}	58.88 ± 25.60^{abcdef}
	Nepgen	81.67 ± 5.20^{cdefgh}	91.67 ± 1.04^{efgh}	55.88 ± 7.71^{abcde}	0.89 ± 0.37^{a}	0.62 ± 0.26^{a}	1.52 ± 0.63^{a}	3.46 ± 0.40^{ab}	15.96 ± 8.06^{ab}
	Ofer	69.17 ± 3.82^{abcd}	93.85 ± 0.75^{fgh}	64.24 ± 16.73^{abcde}	1.11 ± 0.22^{a}	0.78 ± 0.15^{a}	1.89 ± 0.37^{a}	4.80 ± 0.59^{ab}	21.31 ± 14.73^{abc}
	Robusta	97.32 ± 0.38^{h}	91.65 ± 0.44^{efgh}	61.16 ± 25.48^{abcde}	42.62 ± 8.79^{c}	29.84 ± 6.15^{c}	72.46 ± 14.94^{c}	6.06 ± 2.83^{b}	7.44 ± 4.48^{a}
Chutney	Gymno C	68.33 ± 3.82^{abc}	94.55 ± 0.62^{fgh}	41.99 ± 10.16^{abcd}	0.86 ± 0.06^{a}	0.60 ± 0.04^{a}	1.46 ± 0.10^{a}	1.27 ± 0.16^{a}	95.41 ± 2.80^{fg}
	Meyers	66.67 ± 5.20^{abc}	95.30 ± 0.58^{fgh}	35.75 ± 7.54^{abcd}	3.66 ± 1.72^{ab}	2.56 ± 1.20^{ab}	6.22 ± 2.92^{ab}	1.36 ± 0.32^{a}	87.53 ± 0.72^{defg}
	Nepgen	75.83 ± 8.04^{abcdefgh}	98.25 ± 1.00^{h}	18.39 ± 1.16^{ab}	1.52 ± 0.50^{a}	1.07 ± 0.35^{a}	2.59 ± 0.85^{a}	0.51 ± 0.29^{a}	69.11 ± 17.46^{bcdef}
	Ofer	72.50 ± 2.50^{abcde}	94.57 ± 0.56^{fgh}	27.22 ± 5.43^{abc}	0.65 ± 0.10^{a}	0.45 ± 0.07^{a}	1.10 ± 0.17^{a}	0.66 ± 0.26^{a}	93.26 ± 1.95^{fg}
	Robusta	94.29 ± 0.66^{efgh}	97.34 ± 0.15^{h}	37.98 ± 6.98^{abcd}	6.38 ± 0.75^{ab}	4.47 ± 0.53^{ab}	10.85 ± 1.28^{ab}	1.89 ± 0.65^{ab}	47.91 ± 7.93^{abcdef}
Dried	Gymno C	79.17 ± 3.82^{bcdefgh}	94.61 ± 1.12^{fgh}	78.65 ± 28.3^{cde}	2.51 ± 0.75^{ab}	1.76 ± 0.52^{ab}	4.27 ± 1.27^{ab}	81.92 ± 2.83^{de}	87.78 ± 9.52^{defg}
	Meyers	95.20 ± 0.13^{efgh}	95.63 ± 1.21^{fgh}	66.87 ± 31.53^{bcde}	4.57 ± 2.75^{ab}	3.20 ± 1.93^{ab}	7.77 ± 4.68^{ab}	81.27 ± 0.56^{de}	100.96 ± 59.49^{fg}
	Nepgen	85.00 ± 9.01^{cdefgh}	93.33 ± 3.63^{efgh}	63.23 ± 15.88^{abcde}	0.33 ± 0.23^{a}	0.23 ± 0.16^{a}	0.56 ± 0.38^{a}	78.72 ± 2.76^{d}	78.52 ± 19.92^{cdefg}
	Ofer	74.17 ± 7.22^{abcdefg}	94.66 ± 1.48^{fgh}	47.58 ± 18.25^{abcd}	1.65 ± 0.72^{ab}	1.16 ± 0.50^{ab}	2.81 ± 1.22^{ab}	83.31 ± 3.50^{e}	89.23 ± 8.81^{efg}
	Robusta	96.46 ± 1.21^{gh}	94.71 ± 0.17^{fgh}	109.46 ± 27.80^{e}	42.47 ± 12.81^{c}	29.73 ± 8.97^{c}	72.20 ± 21.78^{c}	72.78 ± 3.20^{c}	126.82 ± 39.66^{g}
Juice	Gymno C	73.33 ± 14.65^{abcdef}	88.37 ± 5.38^{defg}	33.07 ± 17.67^{abcd}	0.59 ± 0.11^{a}	0.42 ± 0.08^{a}	1.01 ± 0.19^{a}	1.07 ± 0.01^{a}	59.56 ± 11.97^{abcdef}
	Meyers	69.17 ± 15.07^{abcd}	87.67 ± 8.58^{def}	39.12 ± 6.07^{abcd}	0.91 ± 0.48^{a}	0.64 ± 0.34^{a}	1.56 ± 0.82^{a}	1.38 ± 0.13^{a}	47.74 ± 9.00^{abcdef}
	Nepgen	77.50 ± 8.66^{bcdefgh}	85.41 ± 1.08^{de}	35.08 ± 6.41^{abcd}	0.27 ± 0.08^{a}	0.19 ± 0.05^{a}	0.46 ± 0.13^{a}	0.68 ± 0.26^{a}	53.55 ± 4.98^{abcdef}
	Ofer	69.17 ± 2.89^{abcd}	88.65 ± 3.10^{defg}	29.65 ± 2.34^{abc}	0.90 ± 0.22^{a}	0.63 ± 0.15^{a}	1.52 ± 0.37^{a}	0.85 ± 0.11^{a}	52.06 ± 7.89^{abcdef}
	Robusta	95.64 ± 0.90^{fgh}	96.12 ± 0.14^{gh}	37.00 ± 2.78^{abcd}	5.62 ± 1.46^{ab}	3.93 ± 1.02^{ab}	9.55 ± 2.48^{ab}	3.03 ± 0.15^{ab}	54.74 ± 8.64^{abcdef}
Preserves	Gymno C	53.33 ± 6.29^{a}	68.23 ± 2.26^{ab}	19.77 ± 1.13^{ab}	2.65 ± 3.58^{ab}	1.86 ± 2.50^{ab}	4.51 ± 6.08^{ab}	1.62 ± 0.05^{ab}	34.73 ± 1.82^{abcde}
	Meyers	53.33 ± 3.82^{a}	82.37 ± 1.76^{cd}	23.19 ± 12.14^{ab}	0.88 ± 0.11^{a}	0.62 ± 0.08^{a}	1.49 ± 0.19^{a}	1.60 ± 0.04^{a}	31.58 ± 17.56^{abcd}
	Nepgen	79.17 ± 1.44^{bcdefgh}	75.32 ± 2.58^{bc}	33.12 ± 11.91^{abcd}	1.22 ± 0.36^{a}	0.86 ± 0.25^{a}	2.08 ± 0.62^{a}	1.06 ± 0.05^{a}	78.52 ± 13.32^{cdefg}
	Ofer	57.50 ± 15.21^{ab}	63.19 ± 2.51^{a}	43.29 ± 16.66^{abcd}	0.59 ± 0.14^{a}	0.41 ± 0.10^{a}	1.00 ± 0.23^{a}	0.79 ± 0.02^{a}	15.94 ± 9.56^{ab}
	Robusta	91.40 ± 1.68^{defgh}	93.41 ± 0.11^{efgh}	11.67 ± 1.30^{a}	12.31 ± 4.17^{b}	8.62 ± 2.92^{b}	20.93 ± 7.08^{b}	2.69 ± 0.04^{ab}	74.73 ± 14.95^{cdefg}
AVG		76.71	89.85	46.71	5.77	4.04	9.81	17.54	59.89
STD		14.14	9.05	27.02	11.60	8.12	19.71	31.33	34.57
CV		18.43	10.07	57.86	200.95	200.95	200.95	178.61	57.72
Significance level		p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001

Means with different superscripts in the same column differ significantly

4.3.1.3 Cladodes

To explain the influence of cultivar and product type on the antioxidant properties of fresh and processed cactus pear cladodes, the mean values for the effects of cultivar and product type on the different antioxidants for fresh and processed fruit (pulp) are indicated in Table 4.5

Dried cladodes had consistensently high values (81.67 – 85. 83%) for Chelating activity, while Chutney (40.00 - 75.00%) and preserves (66.67 – 82.50%) had significantly lower values. Cladode juice and pickles had the lowest chelating activity (16.67 – 48.33% and 7.33 - 48.33%, respectively). No one cultivar could be named as having the best chelating activity in cladodes. Chelating activity in cladode juice (16.67 – 48.33%) was much lower than for fruit juice (85.83 – 96.49%) (Table 4.3) and peel juice (73.33 – 95.64%) (Table 4.4).

Chutney and dried cladodes compared well to fresh cladodes in the % DPPH results, as the values did not differ significantly. Values for juice were only slightly lower, while preserved cladodes had the lowest ability to scavenge radicals. In 3.4.1.4 (Table 3.4), high % DPPH levels were correlated to high Phenolic levels in an individual cultivar (Morado). In processed cladodes, this correlation can be roughly seen when values amongst products are compared, but no individual cultivar could be highlighted. The fact that, as seen in Table 4.5, the % DPPH did not drop significantly after processing cladodes into chutney and dried cladodes, only dropped slightly in juice, preserves and pickles, and having an overall average of 84.07%, indicated that antioxidants in cladodes did not lose their ability to scavenge radicals after processing. This phenomenon was also reported by Jaramillo-Flores *et al.* (2012), who found that the antioxidant capacity in heat treated young cladodes (nopalitos) increased. Fresh carotenoid extract that measured antioxidant activity of 53% increased to 70% after it was immersed in water at 93°C for 30 minutes. Phenolic compound extract increased from 45 % to 54% after the same treatment. The antioxidant activity of the nopalitos increased for both extracts with heat exposure (Jaramillo-Flores *et al.,* 2012). Gallegos-Infante *et al.,* 2009 found low radical scavenging activity in cladodes due to monohydroxylated phenolic compounds that rendered phenols with low activity. This was not seen in this study as cladodes showed high ability to scavenge radicals in fresh cladodes as well as in dried and chutney products.

Ascorbic acid levels in dried cladodes were the highest (182.36 - 282.14 mg/100 g) and the lowest in preserves (14.29 - 28.00 mg/100 g). Robusta had the highest Ascorbic acid values overall. This result is surprising as Robusta had low Ascorbic acid levels in fresh cladodes (24.04 mg/100 g) while Gymno-Carpo (77.40 mg/100 g) and Meyers (67.25 mg/100 g) had the highest values. It may be possible that Ascorbic acid is protected in the Robusta cladode during

processing. Nepgen and Robusta juice had the highest Ascorbic acid content, demonstating higher levels than all other cultivars and fruit or peel juice (> 50 mg/100 g).

Regarding Betalains (Betacyanins and Betaxanthins), the only significantly high cultivar in processed cladodes were Robusta, with Robusta pickles (19.2 mg/kg) the highest value. Cladode chutney and dried cladodes had statistically similar values to fresh cladodes (5.40 - 29.2 mg/kg). Preserves had statistically significant low values (0.11-0.54 mg/kg). Although the Robusta cladodes did not differ significantly from the other cultivars, it had elevated values, such as was found in fresh cladodes (Table 3.4).

Jaramillo-Flores *et al.* (2012), found 229 µg/g Carotenoid content in a fresh cladode (nopalito) extract that increased to 379 µg/g after a heat treatment of 93°. In this study the highest Carotene content was found in dried Gymno-Carpo cladodes (254.75 µg/g) and the lowest in Gymno-Carpo chutney (1.36 µg/g) samples. The values in dried cladodes were exceptionally high (103.87 – 254.75 µg/g) compared to fresh cladodes (6.72 -17.87 µg/g), dried fruit (2.15 – 21.87 µg/g) and dried peel (72.78 – 83.31 µg/g). In fact, the Carotene content in fresh cladodes was only 7% of the value in dried cladodes. Reasons for the observed increase in carotene after processing was summarised by Rickman *et al.* (2007) as higher extraction efficiency, release of protein-bound Carotene during heat treatments, degradation of oxidative enzymes and the loss of soluble solids. The values of all other products were statistically similar; in fact, it was very similar to fresh cladodes (Table 3.4). Gymno-Carpo had the highest Carotene content overall and Robusta had the lowest, while in fresh cladodes, both Gymno-Carpo and Meyers had high results (3.4.3.4). Dried cladodes may thus be a very good source of Carotene.

The high Phenolic content found in fresh cladodes (42.84 – 270.93 mg/kg) was again seen in processed cladode products, in fact, dried cladodes had statistically similar values compared to fresh values (181.11 - 273.46 mg/kg). Phenolic values decreaced in a study by Gallegos-Infante *et al.* (2009) from 180 mg/g GAE in fresh to 41.0 mg/g GAE in dehydrated nopal at 45°C and 3 m/sec air flow. Phenolics also decreased from 1589 to 287 µg/g when Jaramillo-Flores *et al.* (2012) subjected fresh young cladodes (nopalitos) to temperatures up to 93°.

The Phenolic levels for chutney (104.98 – 124.81 mg/kg), juice (73.14 - 102.32 mg/kg) and pickles (91.82 - 147.12 mg/kg) did not statistically differ from fresh and dried cladodes. Preserves had very inconsistent levels (0.44 – 61.61 mg/kg) and were the lowest amongst products. Robusta was the cultivar with the lowest Phenolic content overall (as was found in fresh cladodes) while Meyers had the highest Phenolic content.

The conclusion is made that the best product for antioxidant capacity other than fresh cladodes would be a dried cladode product. In the past years, there has been an increasing interest in developing foods that contain dried cladodes because of the high nutritional value in fresh cladodes (Feugang *et al.,* 2006). Drying cladodes for the purpose of making flour has been investigated recently. Cladode flour could be used in bread products as a flour ingredient (such as in tortillas), or in nutritional supplements, in powder, tablet or capsule form (López-Cervantes *et al.,* 2011). It is important to maintain the original properties of cladodes after drying (Gallegos-Infante *et al.,* 2009). From these results it is clear that dried cladodes would be the product of choice as far as high antioxidant content is considered. Chutney and juice could also be considered to be good products for antioxidant capacity. Chutney could be the second choice over juice as it showed higher values thoughout the products. Cladode chutney also demonstrated higher Ascorbic acid and Carotene contents to fruit- and peel chutney and had similar values in Phenolics, Betalains and % DPPH. Pickles were the most marketable cladode product, but it did not perform well in antioxidant capacity tests, but had high Ascorbic acid and Phenolic levels, while the Carotene levels were similar to fresh cladodes. Preserved cladodes were the product with the least antioxidants and antoxidant capacity.

Determining the best cultivar was not as clear as it was for fruit (4.3.1.1.) and peel (4.3.1.2.). In cladodes, Robusta has the highest Ascorbic acid and Betalain values, but had the lowest Carotene and Phenolic contents. Ofer and Gymno-Carpo were the cultivars that had most consistently high antioxidant contents in cladodes, as it had fairly high Ascorbic acid, Phenolic and Betalain values and the highest Carotene values.

In this study a dried Ofer cladode product was determined to be the best choice in terms of cladode products.

Table 4.5: The effect of cultivar and product type on the antioxidant properties of fresh and processed cactus pear cladodes

Product	Cultivar	Chelating activity %	DPPH %	Ascorbic acid mg/100g	Betacyanins mg/kg	Betaxanthins mg/kg	Betacyanins + Betaxanthins mg/kg	Carotene ug/g	Phenolics mg/kg
Fresh	Gymno C	77.50 ± 2.50^{hijk}	95.53 ± 0.15^{k}	77.40 ± 11.34^{abcd}	3.17 ± 2.24^{abc}	2.22 ± 1.56^{abc}	5.40 ± 3.80^{abc}	17.87 ± 1.30^{bcd}	270.93 ± 117.99^{j}
	Meyers	80.83 ± 2.89^{ijk}	92.74 ± 3.27^{ijk}	67.25 ± 18.65^{abcd}	7.54 ± 1.97^{cd}	5.28 ± 1.38^{cd}	12.82 ± 3.34^{cde}	18.15 ± 7.2^{cd}	257.25 ± 60.00^{j}
	Nepgen	89.17 ± 1.44^{k}	94.49 ± 1.37^{ijk}	26.78 ± 1.01^{ab}	4.74 ± 0.39^{abc}	3.32 ± 0.27^{abc}	8.07 ± 0.66^{abc}	6.72 ± 1.50^{abc}	241.53 ± 32.75^{j}
	Ofer	80.83 ± 7.22^{ijk}	83.77 ± 10.41^{efghi}	58.74 ± 11.14^{abcd}	3.17 ± 2.24^{abc}	16.98 ± 1.26^{f}	20.15 ± 2.78^{ef}	17.87 ± 1.30^{bcd}	239.47 ± 17.12^{ij}
	Robusta	70.00 ± 9.01^{ghij}	92.50 ± 0.91^{ijk}	24.04 ± 7.44^{a}	17.18 ± 7.74^{e}	12.02 ± 5.42^{e}	29.20 ± 13.16^{f}	11.29 ± 3.04^{abcd}	42.84 ± 18.92^{abcd}
Chutney	Gymno C	75.00 ± 6.61^{hijk}	94.98 ± 0.43^{k}	115.81 ± 5.45^{cdef}	5.37 ± 1.31^{abc}	3.76 ± 0.91^{abc}	9.13 ± 2.22^{abc}	1.36 ± 0.18^{a}	120.85 ± 3.45^{defg}
	Meyers	40.00 ± 2.50^{cde}	95.15 ± 0.03^{k}	50.60 ± 6.93^{abc}	5.44 ± 2.57^{abc}	3.81 ± 1.80^{abc}	9.25 ± 4.36^{abc}	5.02 ± 0.27^{abc}	121.85 ± 5.91^{defg}
	Nepgen	46.67 ± 1.44^{def}	93.55 ± 0.26^{ijk}	52.57 ± 8.67^{abcd}	3.65 ± 1.18^{abc}	2.55 ± 0.82^{abc}	6.20 ± 2.00^{abc}	3.57 ± 0.22^{abc}	124.81 ± 2.00^{defg}
	Ofer	62.50 ± 2.50^{fgh}	92.19 ± 0.37^{ijk}	58.17 ± 2.37^{abcd}	8.16 ± 1.98^{cd}	5.71 ± 1.39^{cd}	13.86 ± 3.37^{cde}	3.22 ± 0.17^{ab}	116.16 ± 4.57^{cdefg}
	Robusta	53.33 ± 3.82^{efg}	90.16 ± 1.28^{hijk}	115.23 ± 12.51^{cdef}	8.71 ± 0.39^{cd}	6.10 ± 0.28^{cd}	14.80 ± 0.67^{cde}	4.19 ± 0.72^{abc}	104.98 ± 11.99^{bcdef}
Dried	Gymno C	82.50 ± 2.50^{ijk}	93.10 ± 1.17^{ijk}	182.36 ± 34.07^{fgh}	5.02 ± 0.28^{abc}	3.52 ± 0.19^{abc}	8.54 ± 0.47^{abc}	254.75 ± 4.46^{h}	203.18 ± 17.22^{ghij}
	Meyers	83.33 ± 5.20^{ijk}	89.31 ± 3.19^{ghijk}	222.94 ± 49.40^{hi}	8.80 ± 0.09^{cd}	6.16 ± 0.06^{cd}	14.96 ± 0.15^{cde}	145.56 ± 5.36^{f}	228.89 ± 24.01^{ij}
	Nepgen	85.83 ± 3.82^{jk}	89.54 ± 1.53^{hijk}	159.73 ± 31.93^{efgh}	6.12 ± 2.07^{bcd}	4.28 ± 1.45^{bcd}	10.40 ± 3.51^{bcd}	179.95 ± 8.13^{g}	181.11 ± 18.26^{fghij}
	Ofer	82.50 ± 2.50^{ijk}	94.12 ± 0.30^{ijk}	282.14 ± 31.42^{i}	8.21 ± 0.77^{cd}	5.75 ± 0.54^{cd}	13.96 ± 1.31^{cde}	182.08 ± 8.47^{g}	273.46 ± 30.28^{j}
	Robusta	81.67 ± 1.44^{ijk}	88.67 ± 2.20^{ghijk}	191.57 ± 64.44^{gh}	4.57 ± 0.46^{abc}	3.20 ± 0.32^{abc}	7.76 ± 0.79^{abc}	103.87 ± 1.73^{e}	224.71 ± 23.71^{hij}
Juice	Gymno C	31.67 ± 11.27^{bcd}	92.21 ± 6.60^{ijk}	39.00 ± 9.34^{ab}	0.46 ± 0.06^{ab}	0.32 ± 0.04^{ab}	0.77 ± 0.10^{a}	3.28 ± 0.20^{abc}	79.28 ± 8.68^{abcde}
	Meyers	48.33 ± 8.04^{def}	94.53 ± 2.64^{k}	48.56 ± 7.34^{abc}	0.71 ± 0.14^{ab}	0.50 ± 0.10^{ab}	1.21 ± 0.24^{ab}	9.06 ± 4.18^{abc}	102.32 ± 30.22^{bcdef}
	Nepgen	38.33 ± 11.27^{cde}	93.40 ± 1.99^{ijk}	53.48 ± 8.75^{abcd}	0.24 ± 0.05^{a}	0.17 ± 0.04^{a}	0.40 ± 0.09^{a}	4.57 ± 2.01^{abc}	82.29 ± 6.26^{abcde}
	Ofer	37.50 ± 11.46^{cde}	85.29 ± 1.22^{fghij}	35.16 ± 7.43^{ab}	0.55 ± 0.12^{ab}	0.39 ± 0.09^{ab}	0.94 ± 0.21^{ab}	1.93 ± 0.10^{a}	85.97 ± 11.01^{abcde}
	Robusta	16.67 ± 3.82^{ab}	80.18 ± 4.19^{efg}	52.05 ± 10.36^{abc}	3.64 ± 1.05^{abc}	2.55 ± 0.73^{abc}	6.18 ± 1.78^{abc}	3.33 ± 0.05^{abc}	73.14 ± 27.12^{abcde}
Pickles	Gymno C	7.33 ± 6.43^{a}	70.34 ± 0.99^{bcd}	123.62 ± 13.71^{defg}	0.40 ± 0.20^{ab}	0.28 ± 0.14^{a}	0.68 ± 0.34^{a}	24.38 ± 18.39^{d}	108.82 ± 12.24^{cdef}
	Meyers	28.33 ± 10.10^{bc}	67.97 ± 3.13^{bc}	95.90 ± 26.25^{bcde}	0.66 ± 0.24^{ab}	0.46 ± 0.17^{ab}	1.12 ± 0.40^{ab}	14.75 ± 2.83^{abcd}	131.79 ± 29.03^{defgh}
	Nepgen	48.33 ± 6.29^{def}	82.08 ± 0.11^{efgh}	71.65 ± 27.14^{abcd}	1.38 ± 0.09^{ab}	0.97 ± 0.07^{ab}	2.35 ± 0.16^{ab}	13.26 ± 2.41^{abcd}	123.07 ± 12.66^{defg}
	Ofer	25.83 ± 1.44^{bc}	75.49 ± 0.79^{cde}	111.79 ± 34.53^{cdef}	0.67 ± 0.23^{ab}	0.47 ± 0.16^{ab}	1.14 ± 0.40^{ab}	15.72 ± 1.77^{abcd}	147.12 ± 6.15^{efghi}
	Robusta	18.33 ± 1.44^{ab}	77.75 ± 1.46^{def}	157.69 ± 16.59^{efgh}	11.30 ± 0.95^{d}	7.91 ± 0.67^{d}	19.20 ± 1.62^{de}	11.48 ± 1.01^{abcd}	91.82 ± 2.85^{abcdef}
Preserves	Gymno C	66.67 ± 2.89^{ghi}	61.30 ± 1.37^{ab}	14.29 ± 9.59^{a}	0.09 ± 0.02^{a}	0.06 ± 0.01^{a}	0.15 ± 0.04^{a}	11.74 ± 2.37^{abcd}	61.61 ± 5.32^{abcde}
	Meyers	82.50 ± 2.50^{ijk}	63.47 ± 0.42^{ab}	16.74 ± 6.46^{a}	0.32 ± 0.15^{a}	0.22 ± 0.11^{a}	0.54 ± 0.26^{a}	8.71 ± 0.36^{abc}	41.47 ± 15.62^{abcd}
	Nepgen	75.83 ± 1.44^{hijk}	63.81 ± 2.81^{ab}	25.40 ± 17.70^{ab}	0.42 ± 0.06^{ab}	0.29 ± 0.04^{a}	0.71 ± 0.10^{a}	8.80 ± 0.44^{abc}	12.59 ± 13.40^{ab}
	Ofer	76.67 ± 1.44^{hijk}	57.84 ± 0.74^{a}	17.00 ± 1.45^{a}	0.06 ± 0.06^{a}	0.05 ± 0.04^{a}	0.11 ± 0.10^{a}	13.02 ± 2.05^{abcd}	0.44 ± 0.76^{a}
	Robusta	66.67 ± 1.44^{ghi}	76.68 ± 2.49^{cdef}	28.00 ± 8.16^{ab}	0.15 ± 0.08^{a}	0.11 ± 0.05^{a}	0.26 ± 0.13^{a}	7.54 ± 0.56^{abc}	22.81 ± 5.88^{abc}
AVG		58.69	84.07	85.85	4.03	3.31	7.34	36.90	130.55
STD		24.62	11.88	69.66	4.34	3.96	7.74	64.96	82.33
CV		41.96	14.13	81.14	107.67	119.62	105.41	176.05	63.07
Significance level		p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001	p < 0.001

Means with different superscripts in the same column differ significantly

4.3.2 Combined ANOVA for colour, cultivar and tissue type

Analysis of variance (ANOVA) was done on the data for processed products (Table 4.6) in order to determine the influence of fruit colour, cultivar and tissue type and their interactions on the antioxidant content and capacity. Highly significant differences were observed on most variables (cultivar, tissue type and product type) as well as on the interactions between the variables (cultivar x tissue type, cultivar x product type, tissue type x product type and cultivar x tissue type x product type). Colour seemed to be the only variable that could not consistently and significantly influence the Phenolic, Ascorbic acid or Carotene content. Percentage DPPH and % Chelating activity were significantly influenced by colour ($p < 0.05$). When the interaction of colour x tissue type was analyzed, no significant influence on % DPPH, Phenolics, Ascorbic acid and Carotene was observed, while the % Chelating activity, the combined and individual Betalains were significantly influenced by the tissue type x colour relationship ($p < 0.001$). Furthermore, colour x product type interaction had no influence on the % Chelating activity, Ascorbic acid and Carotene, while a weaker influence was noted on Phenolics. Percentage DPPH, Betacyanins and Betaxanthins were strongly influenced by the interaction of colour x product type ($p < 0.001$).

Table 4.6: Analysis of variance (ANOVA) for the influence of fruit colour, cultivar, tissue type, type of processed product and their interactions on antioxidant properties of processed cactus pear products.

Antioxidant property:	Colour:	Cultivar:	Tissue Type:	Product Type:	Colour X Tissue Type:	Colour X Product Type:	Cultivar X Tissue Type:	Cultivar X Product Type	Tissue Type X Product Type:	Cultivar X Tissue Type X Product Type
% DPPH	*	***	***	***	NS	***	***	***	***	***
% Chelating activity	*	***	***	***	***	NS	***	***	***	***
Phenolics	NS	***	***	***	NS	*	***	***	***	***
Betacyanins	***	***	***	***	***	***	***	***	***	***
Betaxanthins	***	***	***	***	***	***	***	***	***	***
Betacyanins + Betaxanthins	***	***	***	***	***	***	***	***	***	***
Ascorbic acid	NS	***	**	***	NS	NS	***	***	***	***
Carotene	NS	***	***	***	NS	NS	***	***	***	***

NS = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

4.3.3 Principal component analysis (PCA) of product type and cultivar on the antioxidant properties of different cactus pear tissue types

The aim of the PCA is to identify cultivars that are most associated with a specific antioxidant therefore the biplot makes it possible for cultivars and products to be correlated with the antioxidants that it contains.

4.3.3.1 Fruit

In Figure 4.8 the biplot for fruit (pulp) F1 and F2 explained 69.15% of the variability. Carotene, Ascorbic acid, Phenolics and % DPPH were very closely grouped in processed fruit, as was also seen in the PCA on fresh fruit (Figure 3.3). The only products in this grouping were dried Ofer and dried Meyers fruit. Percentage Chelating activity associated with Betalains (Betacyanins and Betaxanthins). This association was also seen before in fresh fruit (Figure 3.3) and products in this bundle were fresh, dried and chutney Robusta products. The products made from the other cultivars were not closely related to any specific antioxidant content or potential.

Herken and Guzel (2010) investigated fruit juice to determine the correlation between Phenolics and antiradical activity. It was thought that the major source of antioxidant capacity in most fruit juices are not from Vitamin C, but from Phenolics. It was found that red fruit (cherries and strawberries) derived antioxidant activity from anthocyanins (red pigment) and phenolics, while in orange nectars it was from ascorbic acid. The current results are in agreement with the work of Herken and Guzel (2010) who established that red products had a different grouping of antioxidants than orange fruit.

Costa *et al.* (2012) found that commercial fruit juice blends that contained high ascorbic acid content values (43.8 mg/100 ml) as well as high phenolic content values (133.0 mg/100 ml GAE), had superior DPPH scavenging activity (308.1 mg/100 ml Trolox). The juice that contained low ascorbic acid (18.8 mg/100 ml) and high phenolics (67.5 mg/100 ml GAE) values, only had almost 50% of the scavenge activity (149.4 mg/100 ml Trolox), while the juice with the lowest DPPH value (29.4 mg/100 ml Trolox) had equally low ascorbic (31.3 mg/100 mg) and phenolic (31.6 mg/10 ml GAE) values. In our findings, both Ascorbic acid and Phenolics had high values in fruit, and were grouped together in the PCA. This could explain the high antiradical activity found in orange cactus pear fruit.

In conclusion, groupings or associations of antioxidants are present in processed fruit, as was seen in fresh fruit, but most of the products did not seem to associate closely with either of the

groups in the red or green ovals. As a matter of fact, all other products were grouped together to the side of % Chelating activity, % DPPH and the antioxidants (blue circle). In the red circle, Ofer, Gymno-Carpo and Meyers dried products had high Ascorbic acid, Phenolics, Carotene and % DPPH values, while in the green circle, dried and fresh Robusta had high Betalain and % Chelating activity. Therefore results from the PCA (Figure 4.8) correlate with Table 4.3.

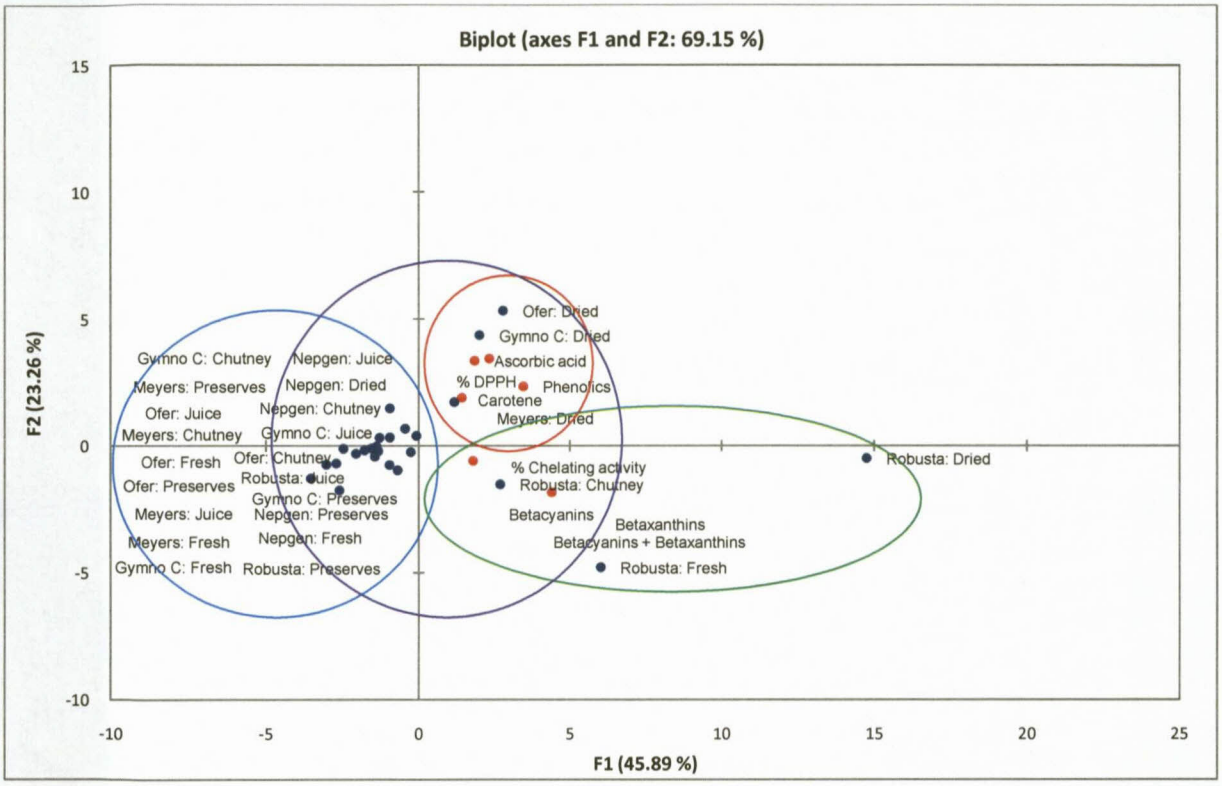


Figure 4.8: Principal component analysis of product and cultivar on the antioxidant properties of cactus pear fruit (pulp)

4.3.3.2 Peel

In Figure 4.9, F1 and F2 explained 72.80 % of the variation. In this figure, it is evident that all the products seem to cluster together and that there is no definite distinction between cultivars or products and their relationship to antioxidants (purple circle). This result could indicate that all products and antioxidants are associated in the peels, more so than in fruit (pulp). Nevertheless it is possible to see that % DPPH, Ascorbic acid, Carotene and Phenolics are grouped together in the upper right quadrant (red circle) with dried peel from Gymno-Carpo, Ofer, Meyer and Nepgen. In the lower left quadrant it seems that preserved products are clustered together and are not closely related to any of the antioxidants (lowest values in Table 4.4) while in the upper left quadrant the chutneys seems to bundle (high values in Table 4.4) (green circle). Robusta

products were scattered around the Betalain content marker (blue circle). Ofer (fresh and juice) (on the left) are not associated with any of the antioxidants.

Overall though, all the antioxidant content and -capacity markers seem to be related to each other and the products are aside but are close enough to associate with them. The PCA figure correlates with data from Table 4.4, since the products with the highest contents and –capacities were situated nearby the associated markers, whereas products with similar results (chutney and preserves) were bundled together.

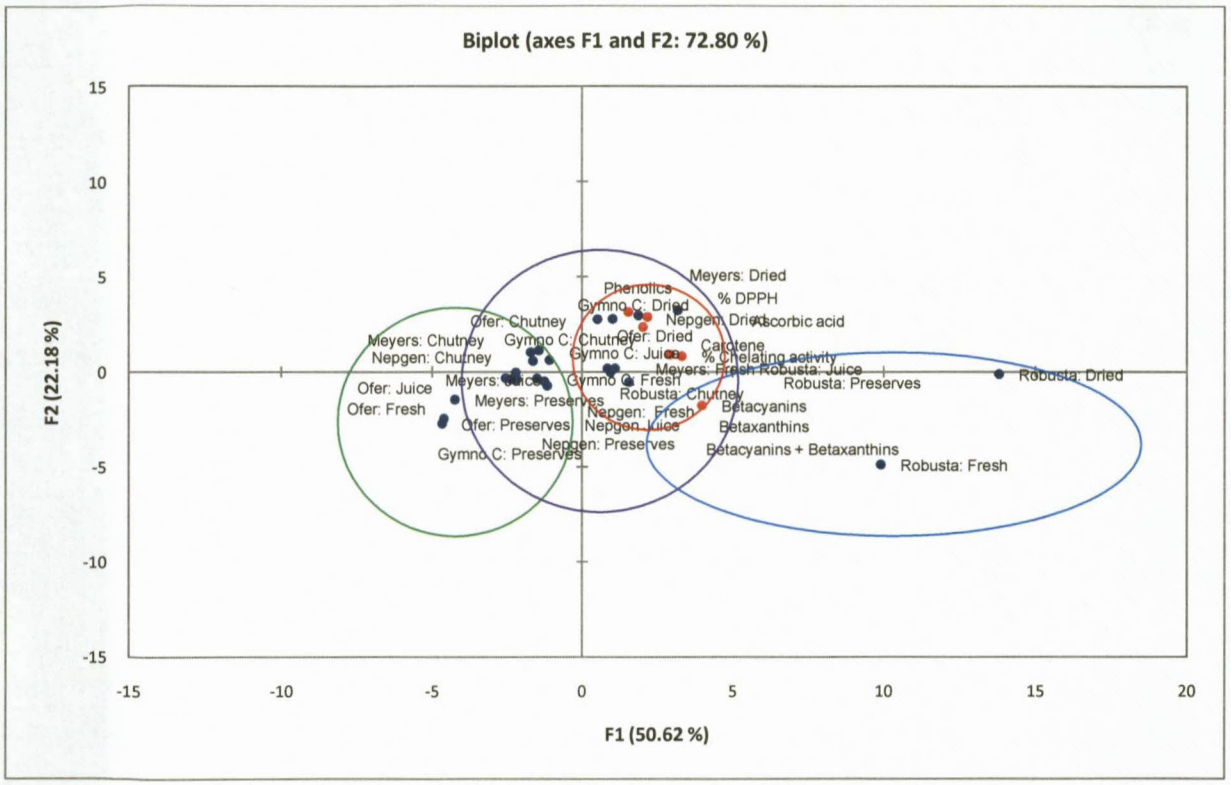


Figure 4.9: Principal component analysis of product and cultivar on the antioxidant properties of cactus pear peels.

4.3.3.3 Cladodes

In the PCA analysis (Figure 4.10) of cladodes (F1 and F2 explained 72,80% of the variability) the data clearly indicated that dried products were closely related to Carotene, Ascorbic acid and Phenolics except for dried Robusta that, together with all other Robusta products were clustered around Betacyanins and Betaxanthins (indicated in the purple oval). Percentage DPPH and % Chelating activity is closely related to each other and the fresh cladodes from Gymno-Carpo, Nepgen and Meyers and chutney from Nepgen and Meyers (these products had the highest results for % DPPH and Chelating activity in Table 4.5). It was not only the products

made from Robusta that were clustered around the Betalains, but Ofer chutney and fresh cladodes from Ofer and Meyers were also clustered in the purple circle. The other products were clustered closely together (green oval), but did not seem to be associated with the antioxidants tested in this study. It is noteworthy that preserves (all cultivars except Robusta) are the on the far left, meaning that preserves had a weak association with all the antioxidants and antioxidant potential results.

It is clear from the PCA on cladodes that the products seem to cluster together, in stead of colour or cultivar, except for Robusta products that are closely associated with Betalains. This conclusion mirrors the findings in the ANOVA variance analysis (Table 4.6).

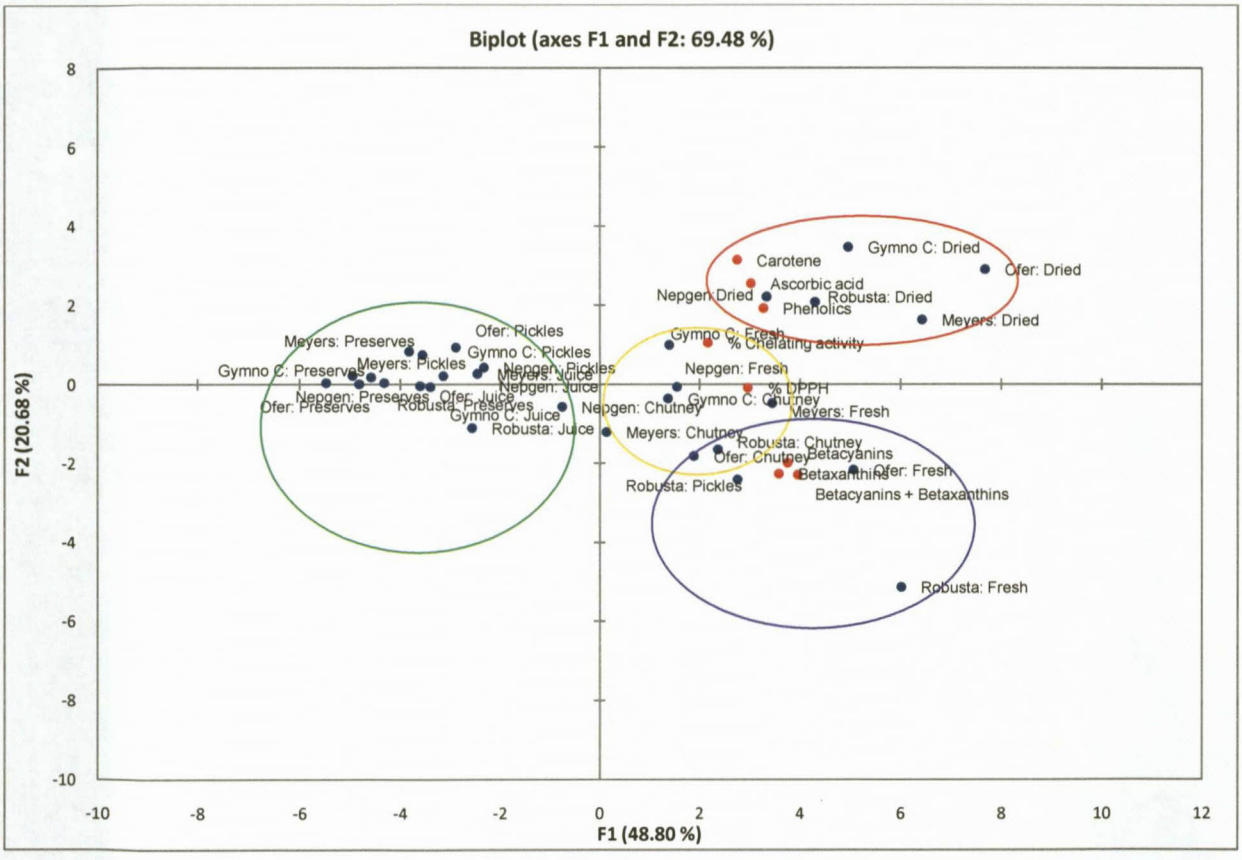


Figure 4.10: Principal component analysis of product and cultivar on the antioxidant properties of cactus pear cladodes.

4.3.4 The effect of colour and product on the antioxidant properties of cactus pear fruit

A main aim in this study was to ascertain whether specific antioxidants occur in coloured cactus pear fruit, therefore Table 4.7 and Figures 4.11 to 4.16 was included to determine whether the colour of the specific cactus pear fruit product had an influence on antioxidant content and capacity. Sacchetti *et al.* (2008) suggested that colour could only be considered as an indicator of the antioxidant potential of foods, if colour and antioxidant has the same formation mechanisms. The results of mean values and the significant values are indicated in Table 4.7. In order to examine the antioxidants (content and capacity) individually in more detail, each product is represented in graphical format according to colour (Figures 4.11 to 4.16).

4.3.4.1 Percentage Chelating activity

In terms of Chelating ability, all the colours and products had results of between 70 and 96% (Figure 4.11). The purple fresh fruit seemed to have the most consistently high levels, with purple preserves (90%) the lowest and purple juice (96%) the highest value. Purple is therefore the colour with the highest % Chelating activity. Pink had lower results than other colours, since juice (85%), chutney (76%) and preserves (70%) had lower values than the other colours. The two best products were fresh fruit (91 – 95%) and fruit juice (85 -96%). The preserves had lower values overall, but only orange (70%) and pink (70%) preserves had significantly lower values. Therefore, the best colour fruit for chelating activity is purple and the best product is juice.

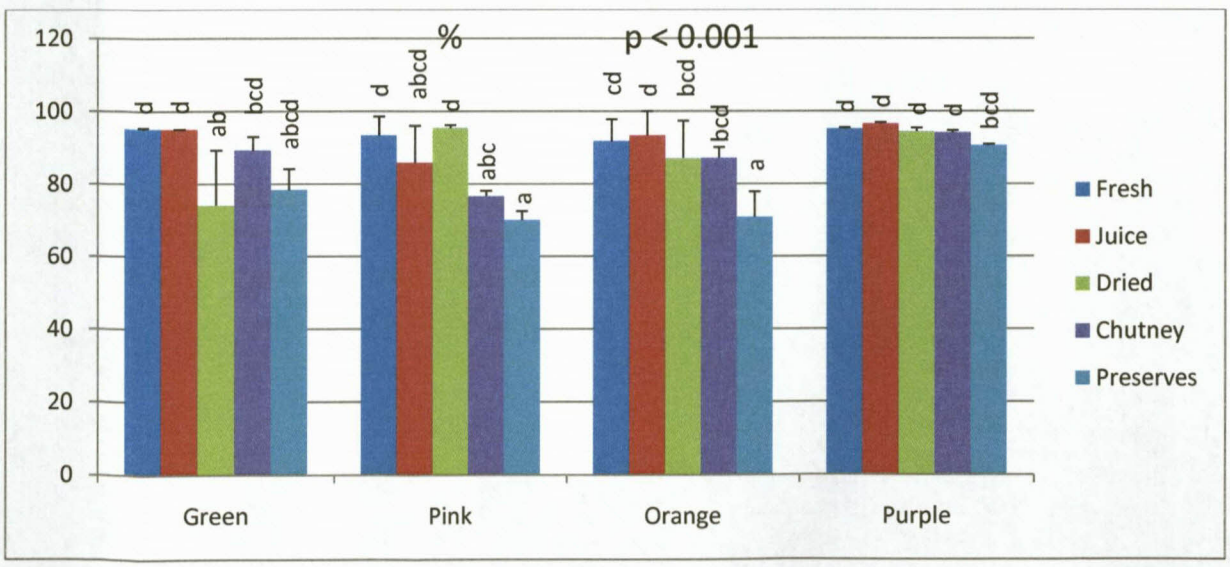


Figure 4.11: The effect of colour and processed product on the % Chelating activity of cactus pear fruit

Table 4.7: The effect of colour and processed product on the antioxidant properties of cactus pear fruit.

Product	Cultivar	Chelating activity %	DPPH %	Ascorbic acid mg/100g	Betacyanins mg/kg	Betaxanthins mg/kg	Betacyanins + Betaxanthins mg/kg	Carotene ug/g	Phenolics mg/kg
Fresh	Green	94.94 ± 0.37^{d}	83.63 ± 1.30^{cd}	91.69 ± 8.98^{cdef}	1.83 ± 0.55^{a}	1.28 ± 0.39^{a}	3.11 ± 0.94^{a}	0.62 ± 0.19^{a}	16.72 ± 7.48^{a}
	Orange	91.67 ± 6.06^{cd}	73.84 ± 5.93^{b}	94.67 ± 23.15^{def}	1.37 ± 0.28^{a}	0.96 ± 0.19^{a}	2.33 ± 0.47^{a}	1.78 ± 0.23^{a}	17.74 ± .91^{a}
	Pink	93.33 ± 5.20^{d}	58.65 ± 11.49^{a}	52.52 ± 5.75^{abcde}	2.71 ± 0.15^{a}	1.90 ± 0.10^{a}	4.62 ± 0.25^{a}	0.92 ± 0.16^{a}	17.45 ± 9.15^{a}
	Purple	95.09 ± 0.38^{d}	77.05 ± 4.53^{bc}	32.38 ± 22.99^{ab}	74.47 ± 41.06^{b}	52.13 ± 28.74^{b}	126.60 ± 69.81^{b}	1.58 ± 0.09^{a}	18.47 ± 6.02^{ab}
Chutney	Green	89.17 ± 3.82^{bcd}	92.02 ± 0.08^{def}	38.62 ± 3.37^{ab}	8.72 ± 3.52^{a}	6.11 ± 2.47^{a}	14.83 ± 5.99^{a}	1.36 ± 0.37^{a}	114.14 ± 22.40^{abcde}
	Orange	87.08 ± 2.92^{bcd}	96.15 ± 1.90^{ef}	62.24 ± 18.20^{abcde}	5.96 ± 2.84^{a}	4.17 ± 1.99^{a}	10.13 ± 4.83^{a}	1.57 ± 2.17^{a}	117.97 ± 21.13^{bcde}
	Pink	76.67 ± 1.44^{abc}	92.06 ± 0.17^{def}	42.49 ± 12.72^{abc}	4.89 ± 0.43^{a}	3.42 ± 0.30^{a}	8.31 ± 0.73^{a}	0.78 ± 0.19^{a}	143.27 ± 4.72^{cde}
	Purple	93.99 ± 0.69^{d}	95.57 ± 0.10^{ef}	15.03 ± 0.53^{a}	31.25 ± 2.78^{a}	21.88 ± 1.95^{a}	53.13 ± 4.73^{a}	0.34 ± 0.11^{a}	150.83 ± 6.11^{cde}
Dried	Green	74.17 ± 15.07^{ab}	98.98 ± 0.11^{f}	83.21 ± 19.27^{bcdef}	1.84 ± 0.26^{a}	1.29 ± 0.18^{a}	3.13 ± 0.44^{a}	2.15 ± 0.27^{a}	121.20 ± 109.32^{bcde}
	Orange	87.08 ± 10.18^{bcd}	92.33 ± 0.26^{def}	142.24 ± 27.14^{g}	3.06 ± 1.16^{a}	2.14 ± 0.81^{a}	5.20 ± 1.97^{a}	30.74 ± 11.04^{c}	183.35 ± 39.78^{e}
	Pink	95.38 ± 0.80^{d}	92.05 ± 0.30^{def}	106.30 ± 40.74^{efg}	5.92 ± 3.54^{a}	4.14 ± 2.48^{a}	10.06 ± 6.01^{a}	4.48 ± 0.65^{a}	175.10 ± 55.12^{de}
	Purple	94.34 ± 1.04^{d}	96.51 ± 0.09^{ef}	113.59 ± 45.53^{fg}	103.58 ± 35.57^{b}	72.51 ± 24.90^{b}	176.08 ± 60.48^{b}	17.14 ± 1.60^{b}	326.33 ± 131.47^{f}
Juice	Green	95.00 ± 0.01^{d}	93.53 ± 0.12^{def}	28.17 ± 6.09^{ab}	0.49 ± 0.07^{a}	0.34 ± 0.05^{a}	0.83 ± 0.12^{a}	0.31 ± 0.19^{a}	77.94 ± 7.85^{abcd}
	Orange	93.33 ± 6.65^{d}	94.61 ± 1.79^{ef}	41.24 ± 5.84^{ab}	0.63 ± 0.13^{a}	0.44 ± 0.09^{a}	1.08 ± 0.22^{a}	0.80 ± 0.24^{a}	73.91 ± 11.80^{abc}
	Pink	85.83 ± 10.10^{abcd}	96.10 ± 0.57^{ef}	48.48 ± 9.62^{abcd}	1.08 ± 0.28^{a}	0.76 ± 0.20^{a}	1.84 ± 0.48^{a}	0.74 ± 0.07^{a}	96.33 ± 19.81^{abcde}
	Purple	96.49 ± 0.33^{d}	94.69 ± 1.45^{ef}	21.21 ± 5.20^{a}	5.46 ± 1.39^{a}	3.82 ± 0.98^{a}	9.27 ± 2.37^{a}	2.01 ± 0.44^{a}	58.97 ± 19.79^{abc}
Preserves	Green	78.33 ± 5.77^{abcd}	86.89 ± 7.10^{cde}	22.43 ± 5.76^{a}	0.28 ± 0.11^{a}	0.19 ± 0.08^{a}	0.47 ± 0.18^{a}	0.27 ± 0.02^{a}	54.50 ± 16.75^{abc}
	Orange	70.83 ± 7.01^{a}	70.57 ± 4.23^{b}	27.48 ± 9.18^{a}	0.41 ± 0.20^{a}	0.29 ± 0.14^{a}	0.70 ± 0.33^{a}	0.61 ± 0.08a	66.35 ± 13.19^{abc}
	Pink	70.00 ± 2.50^{a}	90.29 ± 1.84^{def}	27.44 ± 10.94^{a}	0.96 ± 0.08^{a}	0.67 ± 0.06^{a}	1.63 ± 0.13^{a}	0.93 ± 0.08^{a}	86.75 ± 10.73^{abcde}
	Purple	90.50 ± 0.35^{bcd}	93.56 ± 0.22^{def}	17.02 ± 5.53^{a}	9.99 ± 1.29^{a}	6.99 ± 0.91^{a}	16.99 ± 2.20^{a}	1.76 ± 0.08^{a}	68.04 ± 3.74^{abc}
AVG		87.33	87.86	59.05	11.05	7.74	18.79	4.26	97.79
STD		10.01	11.00	41.39	25.93	18.15	44.07	9.00	77.01
CV		11.47	12.52	70.08	234.56	234.56	234.56	211.42	78.75
Significance level		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

Means with different superscripts in the same column differ significantly

4.3.4.2 Percentage DPPH

In Figure 4.12 the effect of colour and products indicated that processed cactus pear products of all colours demonstrated an excellent ability to scavenge radicals. The highest value was for dried green fruit (98%) and the lowest for orange preserves (70%). All processed products had statistically similar values (92.02 – 98.98%); only orange preserves had lower results that were significantly different. Antiradical activity (% DPPH) was higher than for other dried fruits, such as agen prunes (76.0 %), prunes (72.6%), apricots (61.2%), raisins (78.4%) and figs (28.1%) (Ouchemoukh *et al.*, 2012). Purple was the best colour overall by slight margins and orange the lowest for processed products. Thus, processing purple products would result in cactus pear products with the highest ability to scavenge radicals.

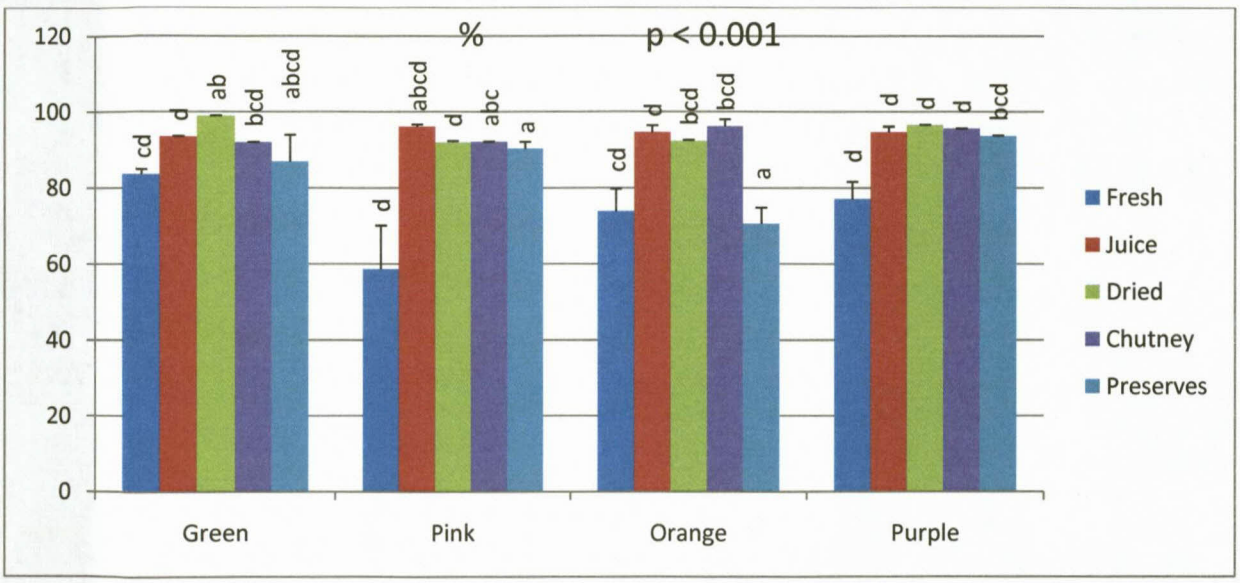


Figure 4.12: The effect of colour and processed product on the % DPPH of cactus pear fruit

4.3.4.3 Betalains (Betacyanins and Betaxanthins)

The purple fruit had much higher Betalain (Betacyanin and Betaxanthin) contents than any of the other colours or processed products (Figure 4.13). Of these purple cultivars, fresh Robusta fruit has been seen to contain up to three times more Betalains than any other cultivar (Table 3.1); in fact, the fresh- and dried fruit of robusta were excellent sources of Betalains (Table 4.3). Dried purple fruit had the highest value (176.08 mg/kg) and green preserves the lowest (0.47 mg/kg). This data correlates with Yahia and Mondragon-Jacobo's (2011) findings that the purple fruit contain more Betalain than the other coloured fruit and the lowest values were measured in the green fruit. Dried Robusta fruit would therefore deliver a product with the highest Betalain content.

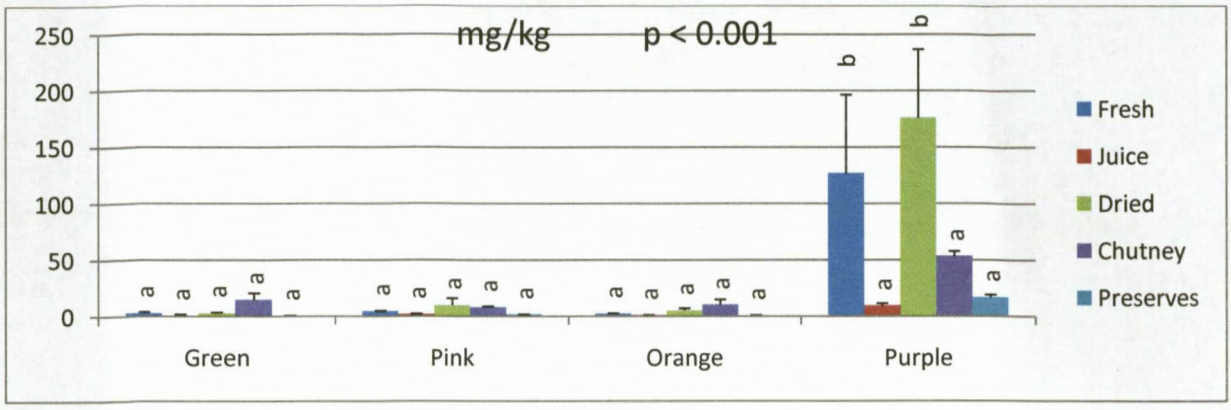


Figure 4.13: The effect of colour and processed product on the Betalain content of cactus pear fruit

4.3.4.4 Ascorbic acid

Colour did not have a significant influence on Ascorbic acid content in the ANOVA analysis (Table 4.6), since neither colour x tissue type, nor colour x product type influenced Ascorbic acid significantly. Yet, in analysis of fruit and fruit products only, the fresh orange and green fruit were determined to be superior to other colours (Chapter 3, Table 3.1). Dried fruit was determined to be the most valuable source of vitamin C (Table 4.3). Similar results were found in Figure 4.14 as the readings for dried fruit (83.21 – 142.24 mg/100 g) were significantly higher than fresh fruit (32.38 – 94.67 mg/100 g), except in green cultivars (91.69 mg/100 g in fresh and 83.21 mg/100 g in dried fruit). Purple products had the lowest Ascorbic acid values (15.03 – 113.59 mg/100 g). All orange coloured cactus pear processed products had the highest content of Ascorbic acid (Figure 4.14) of which dried fruit was the highest. Purple chutney (15.03 mg/100 g) and preserves (17.02 mg/100 g) had the lowest Ascorbic acid content.

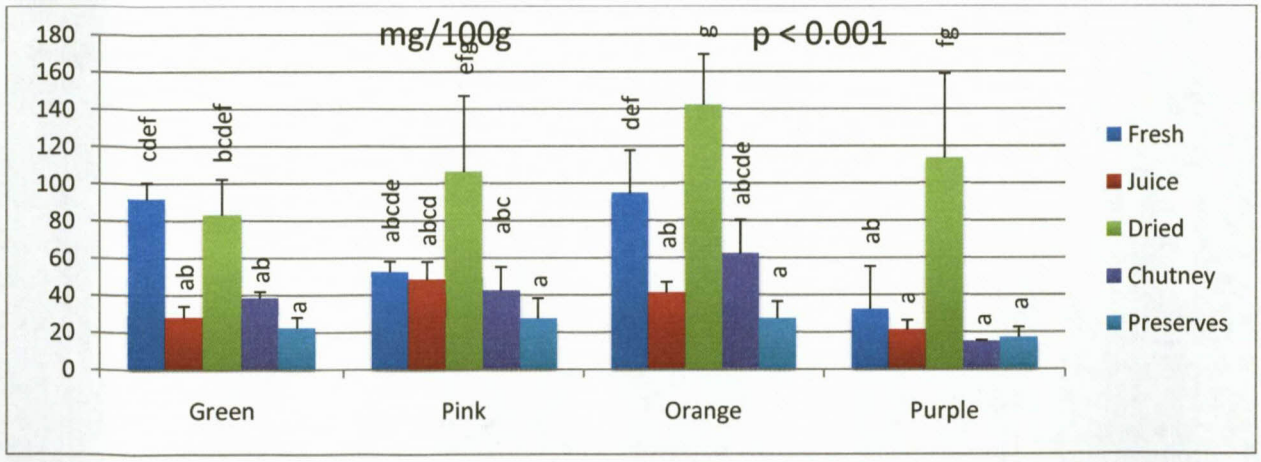


Figure 4.14: The effect of colour and processed product on the Ascorbic acid content of cactus pear fruit

4.3.4.5 Carotene

In ANOVA analysis (Table 4.6) it is evident that colour, colour x tissue type and colour x product type did not influence Carotene content significantly. From Figure 4.15 it is evident that dried fruit has the best results for each cultivar tested; in fact, orange (30.74 µg/g) and purple (17.14 µg/g) dried fruit showed significantly high levels in dried fruit, with orange dried fruit almost twice that of purple dried fruit and almost four times that of the pink dried fruit (4.48 µg/g). Dried pink and green fruit were not significantly higher than any of the other products (0.27 – 2.15 µg/g). Green preserves had the lowest Carotene contents (0.27 µg/g). Dried fruit is recommended as a processed product with nutrient density and quality antioxidants (Vinson *et al.*, 2005)

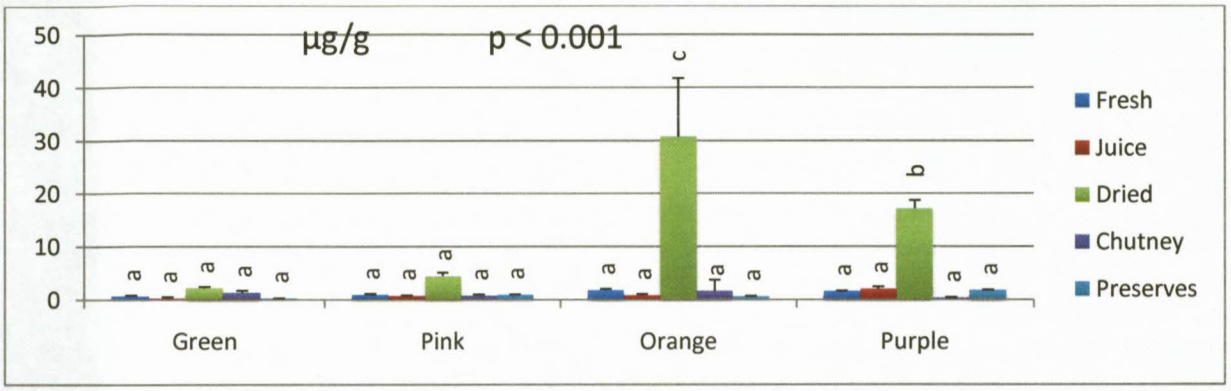


Figure 4.15: The effect of colour and processed product on the Carotene content of cactus pear fruit

4.3.4.6 Phenolics

According to AVOVA analysis (Table 4.6) colour and colour x tissue type did not influence Phenolics significantly. Processed products are better sources of total phenolics than fresh cactus pear fruit (Figure 4.16). Juice, chutney and preserves are not significantly different from each other, but all are higher than the fresh fruit and therefore good sources of Phenolics. Dried fruit products of pink (175.10 mg/kg), orange (183.35 mg/kg) and purple (326.33 mg/kg) fruit are significantly higher than the other products. Chutney (114.14 - 150 mg/kg) and juice (58.97 - 96.33 mg/kg) also showed good contents of Phenolics. In conclusion, in processed cactus fruit, purple dried fruit was the best source of Phenolics, while green preserves (54.50 mg/kg) had the lowest values.

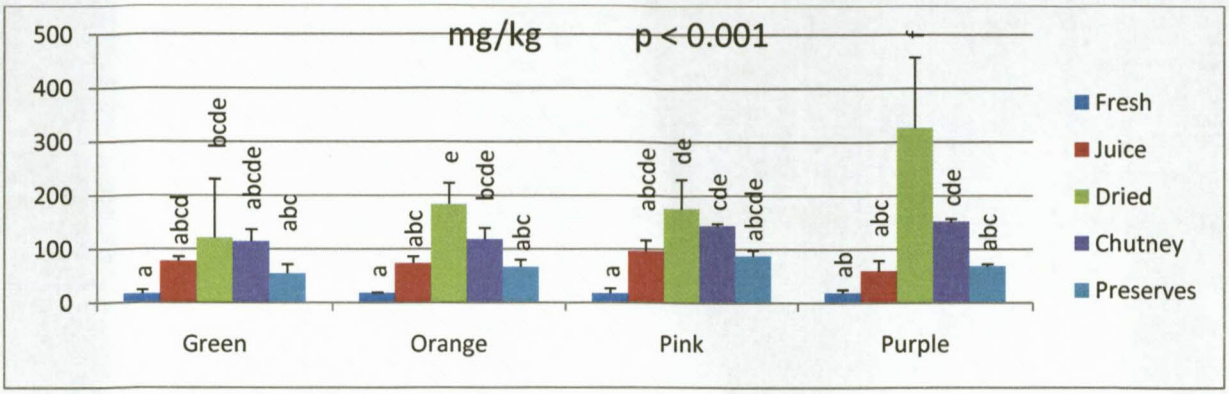


Figure 4.16: The effect of colour and processed product on the Total Phenolic content of cactus pear fruit

4.3.5 Principal component analysis (PCA) of colour and processed product type on the antioxidant properties of fresh cactus pear fruit.

In Figure 4.17 the biplot F1 and F2 explained 69.86% of the variability. The PCA of colour and processed product type (Figure 4.17) were very similar to the principle component analysis of product and cultivar on the antioxidant properties of fresh cactus pear fruit (Figure 4.8). From the PCA for fresh fruit (Figure 3.3) and peel (Figure 3.4) it was clear that associations found between antioxidant content and capacity were as follows: Ascorbic acid, Phenolics and % DPPH group together, while Carotene, Betalain and % Chelating activity associate.

Carotene, Ascorbic acid, Phenolics and % DPPH were very closely grouped in processed fruit as was seen in the PCA on fresh fruit (Figure 3.3) and processed fruit (Figure 4.8). Colours in the red circle grouping were dried orange and dried pink fruit, thus products tend to group together instead of colour or cultivar. Percentage Chelating activity associated with Betalains (Betacyanins and Betaxanthins) in the blue circle. Purple products were in this grouping, thus colour determined this association. The third group in the green circle was not closely related to any specific antioxidant content or potential. The colours in this grouping were green, pink and orange products. These products were fairly close to % Chelating activity and % DPPH.

In conclusion, the same associations are evident that was observed in fresh fruit and processed fruit. However, most of the colours did not seem to associate closely with any specific antioxidants in processed products.

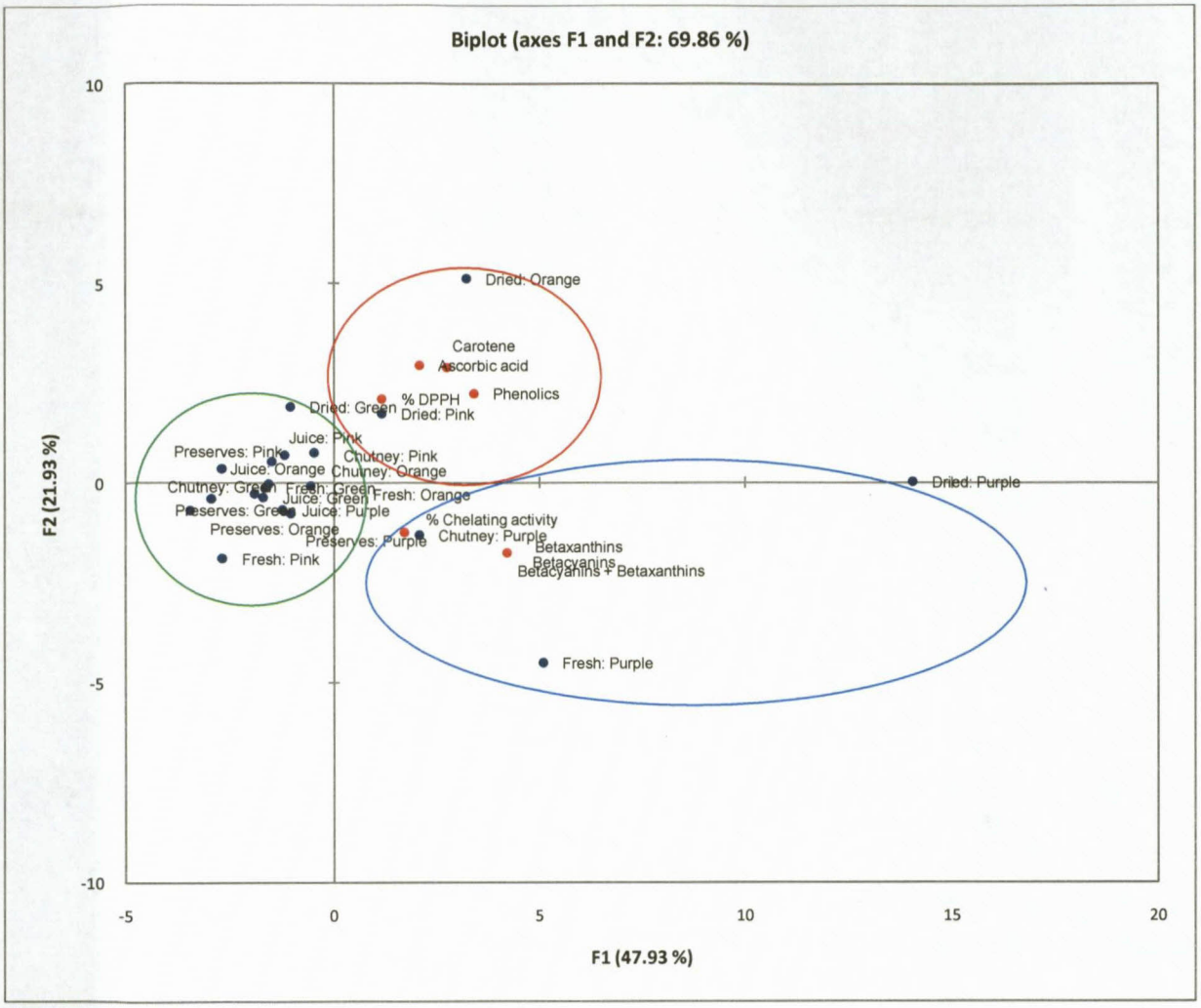


Figure 4.17: Principal component analysis of colour and processed product type on the antioxidant properties of fresh cactus pear fruit.

4.4 Summary of the combined effect of tissue type, colour, cultivar and product on antioxidant content.

Individual antioxidants

Betalains were present in all coloured fruit tested but were significantly higher and far above all other fruit in the purple fresh- and dried fruit of Robusta. It seems as if Betalains were retained well in the dried and chutney products but were lost in the juice and preserves. It is interesting to note that the cladodes of Robusta contained high amounts of Betalains, even though it does not appear to have a purple colour. Morales and Sáenz (2009) found that the degradation of antioxidants after thermal processing was noticeable, but that betalains showed more stability,

therefore purple pulp showed the highest concentration of bioactive compounds after thermal processing.

Orange coloured fruit (Gymno-Carpo and Ofer) had the highest levels of Ascorbic acid. Robusta (purple fruit) had low levels throughout. The normal losses of Ascorbic acid after processing in the juice and chutney were very similar, even though considerably more heat is applied during the making of chutney than in making juice. Ascorbic acid retention has been reported before by Piga *et al.* (2002), Piga *et al.* (2003) and by Miller and Rice-Evans (1997) in blackcurrant, orange and apple fruit juices. It was suggested by the authors that Phenolics protect Ascorbic acid against oxidative decomposition during preservation under mild oxidative conditions. Ascorbic acid was also retained in the pickling process of cladodes. Manufacture of preserved fruit by using whole fruit required intense heat applied for a period of time while the fruit was cooked in syrup; therefore more Ascorbic acid was lost. It can be concluded that fresh and dried cactus fruit products contain the highest levels of ascorbic acid. Piga *et al.,* 2003 found that Ascorbic acid was retained in peeled cactus pear fruit more than in other (persimmons, strawberries and kiwifruit) fruit after minimal processing and storage. Ascorbic acid remained the main antioxidant in cactus pears investigated by Piga *et al.* (2003), even though the contribution of polyphenols was high and fairly consistent. It is speculated that mucilage, present in cactus pear products, might protect antioxidants in processed products.

The results from all the processed fruit and peel products tested for Carotene content were comparable with fresh cactus fruit, as was described in Chapter 3 (3.4.7.3). Cladodes contained higher levels of carotene than fruit. The data reflects that the pulp of the fruit has negligible amounts of Carotene, the peel has slightly more, but the levels in the cladodes are high. The conclusion can be made that Carotene is well preserved in processed cactus pear fruit products. In cladodes, Carotene levels were higher in processed product than fresh cladodes, therefore this study agrees with the findings by Rickman *et al.* (2007) and the theory by Jaramillo-Flores *et al.* (2003) that mucilage in cladodes interferes with carotene determination and that heat treatments allows carotene to be released from the cell matrix.

Phenolic levels of processed products were higher than in fresh fruit and peel. Phenolic levels were higher in cladodes than in fruit and peel as was described in Chapter 3 (3.4.7.4). Fresh cladodes had the highest levels and drying was the best process for preserving Phenolics. Thus, fresh or dried cladodes would be the choice for high Phenolics content in the cactus pear plant.

Antioxidant capacities

Antioxidant capacity in processed cactus pear fruit, peel and cladode products were exceptionally high, in fact some processed products were comparable to or higher than the fresh counterparts (Table 4.3). This was seen before in studies concerning processed fruit by Rickman *et al.* (2007), Ryan and Prescott (2010) and Jaramillo-Flores *et al.* (2003).

Tissue type

Peel showed similar antioxidant content than fruit, both fruit and peel would contribute to excellent antioxidant capacity; therefore the peel should be included in processed products such as juice, dried fruit and chutneys, where possible.

Though cladodes are not used as food in South Africa, processed cladodes are excellent sources of antioxidants: in fact, processed cladodes are a better source than fresh cladodes probably because mucilage present in cladodes captures antioxidants. These are, however released with the heat treatments required in processing techniques (Jaramillo-Flores *et al.,* 2003).

Colour

Purple fruit, peel and cladodes tested the highest in % DPPH, % Chelating activity, Betalains, Carotene and Phenolics. In orange and pink fruit, peel and cladodes, it was Ascorbic acid that dominated. Purple (first) and orange (second) were therefore the colours of cactus fruit cultivars that could be the best choice in terms of antioxidant content.

Cultivar

The cultivar that could be pinpointed as the best fruit (pulp and peel) for preservation was Robusta. It had high % DPPH and % Chelating activity readings, very high Betalain content and fair amounts of Ascorbic acid, Carotene and Phenolics. Nepgen was the cultivar with the lowest antoxidant content (not significantly), but demonstrated equally good antioxidant capacity levels.

Determining the best cladode cultivar was not as clear as it was for fruit (4.3.2.1.) and peel (4.3.2.2.). In cladodes, Robusta had the highest Ascorbic acid and Betalain content, but had the lowest Carotene and Phenolic content. Ofer and Gymno-Carpo were the cultivars that had the most consistently high antioxidant contents in cladodes, as it had fairly high Ascorbic acid,

Phenolic and Betalain values and the highest Carotene values. Ofer cladode products were determined in this study to be the best choice in terms of high antioxidant cladode products.

Preservation process

Processing had a greater influence on the antioxidant content of cactus fruit and cladode products than the influence of cultivar or colour. Cactus pear juice and chutney are recommended products, but dried (fruit and peel) is the product/processing method of choice regardless of cultivar. According to Vinson *et al.* (2005), dried fruit has greater nutrient density and signifantly more phenol antioxidant content than fresh fruit. Dried fruit has a longer shelf life, contains complex carbohydrates and is high in fibre, therefore dried fruits should be recommended to be added to daily diets. Due to the lifestyle of modern people, convenience foods are becoming more popular. Consequently, dried products are extremely popular because of the light weight, shelf-stability and small size (Sharma *et al.,* 2011).

In terms of colour choice per product, the following would be recommended based according to the data:

- dried products made from purple fruit (Robusta) and cladodes of any cultivar/colour;
- juice should be made from pink or orange fruit;
- for chutney, orange or purple (robusta) fruit should be used;
- pickles from cladodes of any cultivar.

4.5 Conclusion

It should be considered that the stability that Betalains showed in the current study, in regard to heat and pH, are known (Moβhammer *et al.*, 2006b). Ascorbic acid may be retained or protected in certain circumstances during processing (Piga *et al.,* 2003). The increase in Carotene content after processing is documented (Jaramillo-Flores *et al.,* 2003) and lastly the Phenolic levels displayed high levels in the current study. Therefore the possibility must be considered that some processed products such as dried products, juice and chutneys, that are more concentrated than fresh fruit and cladodes, have higher antioxidant contents and –capacities than fresh fruit and cladodes. Consequently, processed products may provide more antioxidants to the consumer than fresh fruit and cladodes.

An important observation from the PCA's (Figures 4.8, 4.9, 4.10 and 4.17) statistics was that antioxidants work in groups in certain colour fruit. In purple fruit, Betalains (Betacyanins and Betaxanthins) were mainly active with some assistance from Carotene. In pink and orange fruit, Ascorbic acid, Phenolics and to a lesser extend Carotene were grouped together. The active antioxidant in green fruit was not clear in any of the investigations in this study. It was reported in previous literature that antiradical activity could be caused by different antioxidants (Herken and Guzel, 2010). It was suggested by Costa *et al.* (2012) that, when Phenolics and Ascorbic acid are both present in large amounts, a synergetic effect is seen in terms of antioxidant potency in fruit. It was also seen in the current study that certain antioxidants group together and seem to cooperate in order to achieve exceptionally high antioxidant potential such as is seen in the cactus pear fruit. It is believed that antioxidants that group together, assist each other and may therefore work synergistically in cactus pear fruit, peel and cladode fresh and processed products.

Chaper 5
Concluding discussion

It is evident from the literature that there are different opinions as to which antioxidant and colour provide the highest antioxidant capacity in cactus pear fruit. It is agreed amongst researchers that cactus pear fruit improve the redox state in humans, resulting in improving health. Mostly, red- and purple coloured fruit are reported as the colour fruit containing the highest antioxidant potential. Sumaya-Martinez *et al.* (2011) suggested that Phenolics and Ascorbic acid caused red coloured fruit to be the cactus fruit colour with the highest antioxidant capacity. Stintzing *et al.* (2005) suggested Phenolics as the major contributor, then Betalains and lastly Ascorbic acid. Kuti (2004) observed that the high antioxidant capacity in purple fruit may be contributed by Phenolics or by a synergistic effect amongst lesser known antioxidants. Tesoriere *et al.* (2004) simply stated that something other that Ascorbic acid was at work and therefore Betalains must be responsible. Yahia and Mondragon-Jacobo (2011) suggested that the combination of Betalain, Ascorbic acid and Phenolics contributed more than Carotene.

It was concluded from the current data that *Opuntia rubusta* Robusta showed the highest antioxidant content and -capacity and therefore purple fruit from *Opuntia robusta spp.* was the cactus pear that would be most related to high antioxidant capacity. In purple fruit, the high content of Betalains was the antioxidant contributing the antioxidant potential in combination with low levels of Carotene. Other colour fruit contained very low levels of Betalains and thus it cannot be the contributor in orange, pink or green fruit.

Both the orange cultivars (*Opuntia ficus-indica* Gymno-Carpo and Ofer) had exceptional, but lower antioxidant capacities than purple (Robusta) in this study. In orange cactus pear fruit it was found that Ascorbic acid, Phenolics and to a lesser degree Carotene cooperate to provide the antioxidant capacity. It could be speculated that these antioxidants are the most dominant in *Opuntia ficus-indica spp.* fruit in general and that this combination would work in cooperation in pink, purple and orange fruit from *O. ficus-indica*. The contributor of antioxidant capacity in green fruit could not be established in this study.

Cactus pear fruit seeds, and thus products such as cactus pear seed oil may contain high levels of antioxidants. Further study is necessary in order to determine antioxidant potential in cactus seed oil. Cladodes of *O. ficus-indica* had higher antioxidant contents than *O. robusta*, but among the different cultivars, there were few differences.

The heat associated with food processing changed the antioxidant contents, but it was established in the second part of the study that antioxidants remained present and in some cases, the levels improved in cactus pear products to such an extent that it might be classified as being a nutraceutical product. The fruit and peel should be processed into marketable products such as juice, dried fruit, chutney and preserves, while cladodes should be introduced to the South African market, dried and incorporated in other products as flour or preserved into pickles.

The current *Opuntia spp.* pharmacological profile include the antioxidant capacity, analgestic action, anti-inflammatory properties, antiulcerogenic effect, hypoglycemic and antidiabetic effects, anti-hyperlipidemic, cholesterol lowering, anti-arhergenic, diuretical and many more health benefits (Stintzing *et al.* 2005). Livrea and Tesoriere (2006) proved its effectiveness against cancerous tumor growth. The benefits of including cactus pear products in the South African diet are therefore clear and its use should be stressed amoungst consumers.

There has been increased interest for products derived from the cactus pear plant due to their potential nutraceutical effects. Compounds found in these products are known to combat oxidative stress and chronic diseases (Chavez-Santoscoy *et al.*, 2009). Fresh fruit and cladodes are only ready to be harvested for a few months of the year and storage time is limited. Food technologists are challenged to develop procedures to lengthen storage life of the cactus pear fruit and cladodes and to diversify by producing different preserved products (Sáenz *et al.,* 2004; Joubert, 1993; Piga *et al.,* 2003). Several products could be obtained from processing the fruit and cladodes. Some of the traditional and known products are juice, marmalades, jellies, jams, dried sheets, pickles, candy and alcoholic drinks. Recently developed products include sweeteners, frozen fruit, cladode flour, oil (from the seeds), mucilage (from cladodes), pigments and dietary fibre (from the cladodes) (Sáenz, 2000).

In South-Africa, the *Opuntia ficus-indica* plant is mainly cultivated for the fruit destined for the local and European markets. There is currently a small but well developed commercial sector in South-Africa, but the plant as a whole is mainly under-utilized and under-valued. It is only through research such as this study that the true value of this easily cultivated plant may be realized as a healthy food resource. If the *Opuntia ficus-indica* plant may find applications in the food industry, other than that of fresh fruit, by means of the by-products of the fruit such as the peel, the seeds or the cladodes, a larger market may emerge that the South African farmers could supply and thus making it a more economically viable crop.

Summary

Different coloured cactus fruit, peel, seeds and cladodes were studied for antioxidant content and –capacity in fresh products. Fruit and cladodes from seven different cultivars from the *O. ficus-indica spp.* and one of *O. robusta spp.* were collected representing the four colours of fruit available namely, green, pink, orange and purple.

It was found that fresh cactus pear fruit and cladodes contained exceptionally high levels of Ascorbic acid, Total Phenolics and Betalains and modest amounts of Carotene. Antioxidant potential remained at very high levels regardless of the specific antioxidant content. The highest antioxidant content and –capacity were found in purple (*O.robusta* Robusta) fruit and cladodes, attributed to the high levels of Betalains. Ascorbic acid, working synergistically with Phenolics, was found to provide almost as much antioxidant capacity to orange fruit coloured cultivars. It was thus found that cultivars with purple and orange fruit and cladodes were the best in regards to antioxidant content as well as -potential.

Five cultivars, representing the four colours were further investigated by processing into different products in order to determine the influence that preservation techniques had on the antioxidant content and potential in the fruit, peel and cladodes. Juice, dried products, chutneys, whole preserves and pickles were prepared from the fruit (pulp), peel and cladodes of the five different coloured cultivars that attained the highest values in the fresh study.

Betalains were retained in processed products; Ascorbic acid was mostly preserved in the processed products that involved minimal heat treatments, while Carotene and Phenolics increased after processing. Processed cladodes, more than fresh cladodes, from all the cultivars, were concluded to provide an excellent source of antioxidants and could be suggested for products such as cladode flour and pickles. The peel in general was found to contain very similar antioxidant content and potential as the fruit and should be included in products when possible. In fruit, the purple fruit is highly recommended for processing, as it displayed the highest antioxidant potential in its fresh form and maintained these levels in processed product. Dried fruit is the product with the highest source of antioxidants to the consumer. Juice and chutney from pink or orange fruit would also provide products that the South African consumer is accustomed to with exceptionally high antioxidant potential.

The study brings to light the potential that cactus pear products have for the food industry. It could be developed into a profitable industry if the public could be made aware of the health benefits that they provide.

Opsomming

Verskillende kleure turksvy vrugte, skille, sade en kladodes was bestudeer in hierdie studie vir antioksidant inhoud en -kapasiteit. Vrugte en kladodes van sewe verskillende *Opuntia ficus-indica* kultivars en een *O. robusta* kultivar, wat die vier vrug kleure (groen, pienk, oranje en pers) insluit, is gebruik.

Die bevindinge dui daarop dat vars turksvy vrugte en kladodes, baie hoë vlakke van askorbiensuur, fenole en betalaiene asook laer karoteen vlakke bevat, maar die antioksidant potensiaal bly hoog ongeag die spesifieke antioksidant waardes. Die heel hoogste antioksidant inhoud en -potensiaal vlakke is gevind in die pers (*O. robusta* Robusta) kultivar, in die vrugte sowel as die kladodes. Hierdie hoë vlakke is toegeskryf aan die hoë betalaien inhoud. Askorbiensuur, in samewerking met fenole, het die antioksidant potensiaal vlakke aan die ander kleure en veral aan oranje vrugte verskaf. Daar is dus gevind dat pers en oranje kultivars die beste keuse is vir antioksidant inhoud en -waarde.

In die verdere studie is vyf kultivars, wat steeds die vier turksvy kleure verteenwoordig, verwerk in verskillende produkte sodat bepaal kan word of die antioksidante steeds teenwoordig sal wees na prosessering in die vrugte, skille en kladodes. Sap, gedroogde produkte, blatjang, heel ingelegde produkte en piekels (suurtjies) was voorberei van die vrugte, skille en kladodes van die vyf kultivars wat die beste resultate gelewer het in die eerste deel van die studie.

Daar is gevind dat betalaiene behoue gebly het, askorbiensuur was meestal behou in die prosesse waar minder hitte toegevoeg is, terwyl karoteen en fenole toegeneem het tydens prosessering. Geprosesseerde kladodes, eerder as vars kladodes, van enige kultivar is 'n uitstekende bron van antioksidante en daarom word dit hoogs aanbeveel vir die gebruik in produkte soos turskvy kladode meel en piekels. Die skil, oor die algemeen, het dieselfde hoë antioksidant inhoud as die vrug en behoort ingesluit te word in produkte waar moontlik. Veral die pers vrug word aanbeveel aangesien dit die hoogste antioksidant inhoud gehad het en die inhoud het hoog gebly na prosessering. Gedroogte vrugte is 'n uitstekende bron van antioksidante aan die verbruiker. Turksy vrugtesap en blatjang het ook hoë potensiaal as produkte wat die Suid Afrikaanse verbruiker ken.

Hierdie studie het die hoë potensiaal wat turksvy produkte aan die voedsel industrie kan lewer uitgelig. Dit kan ontwikkel word tot 'n winsgewende industrie as die publiek bewus gemaak kan word van die gesondheidsvoordele wat dit inhou.

Key Terms

Cactaceae, Semiarid plants, *Opuntia, Opuntia ficus-indica, Opuntia robusta,* Cactus pear, Prickley pear, cactus fruit, cladodes, cactus pads, natural antioxidants, antioxidant content, antioxidant capacity, chelating activity, betalains, ascorbic acid, phenolics, carotenes, Trolox equivalent antioxidant capacity (TEAC), processing, preserved fruit.

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