Development and characterisation of a functional beverage from red-fleshed Japanese plums (*Prunus salicina L.)*

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Thesis presented in partial fulfilment of the requirements for the degree of

Master of Science in Food Science

Stellenbosch University

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March 2011

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Signed: Naomi Steyn
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Abstract

Nectar formulations containing red-fleshed plum pulp and varying amounts of red-fleshed plum skin extract were developed. Red-fleshed plum nectar formulations containing 0, 8, 16, 24, and 32% skin extract were benchmarked against twenty-two commercial beverages containing red, violet and blue fruits. The total soluble solid content, pH, titratable acidity, colour, total polyphenolic, individual polyphenolic, total anthocyanin, and ascorbic acid contents, as well as antioxidant activity (oxygen radical antioxidant capacity (ORAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and ferric reducing/antioxidant power (FRAP)) of the commercial beverages and plum formulations were determined. The plum nectar formulations had similar or higher total polyphenolic content, antioxidant activity, and colour values than the average for the commercial beverages. The individual polyphenolic compounds analysed in the nectar formulations were cyanidin-3-glucoside, cyanidin-3-rutinoside, quercetin-3-glucoside, quercetin-3-rutinoside, quercetin-3-xyloside, and neochlorogenic acid. Increasing polyphenolic content and antioxidant activity was observed with an increase in skin extract content of the formulations. The sensory attributes of the formulations were plum and plantlike aroma, plum and plantlike flavour, sweetness, acidity, and astringency. Increases in plantlike aroma and flavour, acidity, and astringency in conjunction with decreases in plum aroma, plum flavour, and sweetness extract were observed with an increase in skin extract. Consumer analysis indicated that all formulations were acceptable. The 0 and 16% skin extract formulations were most preferred, while the 32% skin extract formulation was least preferred. A shelf-life study was conducted in two phases. In Phase 1, the 0, 16 and 24% skin extract formulations were stored at 0 and 5°C (analysis time points: before pasteurisation, after pasteurisation (week 0), and after 1, 2, 4, 6, 8, 12, 16, 18, 20, and 24 weeks of storage). Chemical analyses conducted included colour, total polyphenolic, individual polyphenolic compound, and total anthocyanin contents, and antioxidant activity ($DPPH^{\bullet}$ scavenging activity). Results from Phase 1 indicated close associations between the 16 and 24% skin extract formulations, and between these formulations and all chemical attributes. Regression analysis of results indicated significant ($P \leq 0.05$) decreases in red colour, total anthocyanins, cyanidin-3-glucoside, cyanidin-3-rutinoside, $DPPH^{\bullet}$ scavenging activity, total polyphenolic content, quercetin-3-rutinoside, and quercetin-3-xyloside for formulations stored at 0°C. The total and red colour, total anthocyanins, cyanidin-3-glucoside, and cyanidin-3-rutinoside in formulations stored at 5°C showed similar results. During Phase 2 of the shelf life study, sensory analysis was conducted on the 0 and 24% skin extract formulations stored at 5°C (preparation time points: 0, 1, 2, and 3 months). Sensory attributes, including plum, plantlike, and raisin flavour, sweetness, acidity, and astringency, were stable during storage. Chemical results from Phase 2 were similar to those of Phase 1. The chemical and sensory stability of formulations after 24 weeks of storage in Phase 1 and Phase 2 indicated that, with the exception of the anthocyanin degradation, the formulations could be beneficial to juice industries. Thus, red-fleshed plum nectars have the potential to compete with high-antioxidant fruit beverages.

Opsomming

Nektarformulasies wat rooivleis pruimpulp en varieërende hoeveelhede rooivleis pruimskilekstrak bevat, is ontwikkel en ondersoek. Rooivleis pruimnektarformulasies wat 0, 8, 16, 24, en 32% skilekstrak bevat, is vergelyk met twee-en-twintig kommersiële drankies wat rooi, violet en blou vrugte bevat. Die totale oplosbare vastestof, totale polifenoliese, individuele polifenoliese, totale antosianien- en askorbiensuur inhoude, sowel as die pH, titreerbare suurheid, kleur, antioksidant aktiwiteit (suurstofradikale antioksidantkapasiteit (*ORAC*), 2,2-difeniel-1-pikrielhidrasiel (*DPPH*) radikaal blussingsaktiwiteit, en ysterreduserende/antikoksidantkrag (*FRAP*)) van die kommersiële drankies en pruimnektarformulasies, is bepaal. Die pruimnektarformulasies het soortgelyke of hoër totale polifenoliese inhoud, antioksidantaktiwiteit, en kleurwaardes gehad in vergelyking met die gemiddelde vir die kommersiële drankies. Die individuele polifenoliese verbindings wat in die nektarformulasies geanaliseer is, was sianidien-3-glukosied, sianidien-3-rutinosied, kwersetien-3-glukosied, kwersetien-3-rutinosied, kwersetien-3-xylosied, en neochlorogeniese suur. ‘n Toenames in die kleurwaardes, polifenoliese inhoud, en antioksidantaktiwiteit is waargeneem met ‘n toename in skilekstrak is in die formulasies. Die sensoriese eienskappe van die formulasies was pruim- en plantagtige aroma, pruim- en plantagtige geur, soetheid, suurheid, en frankheid. Toenames in plantagtige aroma en geur, suurheid en frankheid, sowel as ‘n afname in pruimaroma, pruimgeur, en -soetheid, is met ‘n toename in skilekstrak waargeneem. Verbruikersanalise het aangedui dat al die formulasies aanvaarbaar was. Die 0 en 16% skilekstrakformulasies was die mees aanvaarbaarste, terwyl die 32% skilekstrakformulasie die minste aanvaarbaar geag is. ‘n Rakleeftydstudie is in twee fases gedoen. In Fase 1 is die 0, 16, en 24% skilekstrakformulasies by 0 en 5°C gestoor (analiseringstydpunte: voor pasturisasie, na pasturisasie (week 0), en na 1, 2, 4, 6, 8, 12, 16, 18, 20, en 24 weke van berging). Chemiese analise wat gedoen is, sluit totale polifenoliese, individuele polifenoliese verbinding, en totale antosianien inhoude, sowel as kleur en antioksidantaktiwiteit ($DPPH^{\bullet}$ blussingsaktiwiteit) in. Resultate van Fase 1 het ‘n nou verband tussen die 16 en 24% skilekstrakformulasies, sowel as tussen hierdie formulasies en hul chemiese kenmerke aangedui. Regressie-analise van resultate het betekenisvolle ($P \leq 0.05$) afnames geïllustreer in rooi kleurwaardes, $DPPH^{\bullet}$ blussingsaktiwiteit, sowel as totale antosianiene, sianidien-3-glukosied, sianidien-3-rutinosied, totale polifenoliese, kwersetien-3-rutinosied, en kwersetien-3-xylosied inhoude van die formulasies wat by 0°C gestoor is. Die totale kleur-, rooi kleurwaardes, sowel as totale antosianien, sianidien-3-glukosied, en sianidien-3-rutinosied in die formulasies wat by 5°C gestoor is, het soortgelyke resultate gegee. Gedurend Fase 2 van die rakleeftydstudie is sensoriese analise op die 0 en 24% skilekstrakformulasies wat by 5°C gestoor is, gedoen (voorbereidingstydpunte: 0, 1, 2, en 3 maande). Sensoriese eienskappe, insluitend pruim-, plantagtige-, en rosyntjiesmake, soetheid, suurheid, en frankheid, was stabiel gedurende berging. Chemiese resultate van Fase 2 was soortgelyk aan dié van Fase 1. Die chemiese en sensoriese stabiliteit van die formulasies na 24 weke van opberging in Fase 1 en Fase 2 nieteenstaande antosianienafname, het aangedui dat die formulasies voordelig kan wees vir die vrugtedrankiebedryf. Dus het rooivleis pruimnektars die vermoeë om met hoë antioksidant vrugtedrankies mee te ding.

Notes

The language and style used in this thesis are in accordance with the requirements of the scientific journal, *International Journal of Food Science and Technology.*

This thesis represents a compilation of manuscripts where each chapter is an individual entity and therefore some repetition between chapters may occur.

Acknowledgements

Special thanks to

- Dr Dalene de Beer, Agricultural Research Council (ARC), Stellenbosch for her amazing patience, sacrifice of time and effort, support, and invaluable input throughout the course of this project;
- Nina Muller, Department of Food Science, Stellenbosch University (SU) for her interest, incredible understanding and support, sacrifice of time and effort, and input during the course of this project;
- Mardé Booyse, ARC - Biometry Unit, Stellenbosch for her help and patience during the statistical analyses of data;
- Hannel Ham & Chris Smith, ARC Stone Fruit Breeding Programme for supplying the plums;
- Dr Chris Hansmann, ARC, Stellenbosch for his assistance with the regulations and nectar preparation, as well as his helpful invaluable input and interest over the course of the project.
- Christiaan Malherbe for his help with the chemical analyses;
- George Dico for his help in pulping and pasteurization of fruit and pulp;
- David Gray, KWV, Paarl for generously providing the grape concentrates needed to prepare the nectar formulations.
- Department of Food Science, SU, for the use of facilities and personnel;
- ARC, Infruitec, for the use of facilities and personnel;
- Sensory and consumer panels for analysing samples;
- NRF for bursaries;
- My incredible family for their constant motivation, support, and love;
- My friends and fellow students for their interest, support, and love

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Chapter 1

Introduction

Polyphenolic compounds are antioxidants that delay or prevent the oxidation of a substrate when present in low concentrations compared to that of the substrate (Halliwell, 1995). The potential health benefits of polyphenolic compounds are being increasingly recognised, as reports indicate that these compounds inhibit the harmful effects of reactive oxygen species, which act as oxidants (Halliwell, 1995). These polyphenolic compounds protect macromolecules, such as proteins, lipids and DNA, from oxidative degradation. Consumption of polyphenolic compounds is associated with decreased risk of chronic diseases, such as heart disease (Chong *et al*., 2010) and cancer (Thomasset *et al*., 2006), as well as neuro-degenerative diseases, such as Parkinson's and Alzheimer's diseases (Aquilano *et al*., 2008). Furthermore, these compounds possibly have antiulcer, antispasmodic, antisecretory, antidiarrhoeal (Carlo *et al*., 1999) and antihepatotoxic properties (Hemingway & Larks, 1988). Flavonoids, one of the major groups of polyphenolic compounds, inhibit low-density lipoprotein and liposome oxidation, while possessing vasoprotective and anticancer properties (Thomasset *et al*., 2006). As polyphenolic compounds have the potential to possess health-promoting properties, fruit breeders and food manufacturers are prompted to research and develop food products that are high in polyphenolic compounds. Fruits and fruit beverages have great potential in this regard.

In South Africa, a variety of fruit beverage types are governed by the Department of Agriculture and Fisheries (1980), namely fresh fruit juice, unsweetened juice, sweetened juice, nectar, squash, and drink. The term fresh juice is reserved for 100% fruit juice with no added preservatives, which should be consumed within 2 h of production. Unsweetened and sweetened juices are permitted to contain preservatives and natural flavourants, ascorbic acid, and/or carbon dioxide, while sweetened juice can also contain natural and/or synthetic sweeteners. Fruit nectars are allowed to contain less than 100% fruit juice with the amount determined by the type of fruit. Fruit drinks typically contain less than 10% fruit juice. No specific regulations are currently mentioned for plum beverages and therefore, beverages from these fruits are classified as from unspecified fruits (Department of Agriculture and Fisheries, 1980).

Fruit beverages are a large and continuously growing market throughout the world (Anon., 2010). In 2009, fruit drinks made up the largest part of the global fruit beverage market (27.8% of total market value). Nectars made up the second smallest segment (16.3%), with vegetable juices making up the smallest segment of the market with only 7.2%. This indicated a major potential for the development of fruit nectars. Market research indicated a steady increase in the global fruit beverage sales between 2005 and 2009. This increase is expected to continue until 2014. In 2009, the total revenue generated by the global fruit beverage market was $69 357.4 billion. Europe accounted for the largest part of the global fruit beverage market (46.6%) in 2009, while North and South America accounted for 36.8% of the market. The European and American markets are

expected to grow by 1% and 6%, respectively, by 2014, increasing the revenue acquired to $26.8 billion and $43.2 billion, respectively (Anon., 2010). Limited figures are available on the African fruit beverage market.

In South Africa there has also been an increase in the production of traditional fruit beverages, and as in the global market, there has been an explosion in the production of fruit beverages labelled as containing high antioxidant fruit ingredients, such as beverages prepared from pomegranates, cranberries, blueberries, and strawberries (Deciduous Fruit Producers' Trust, 2009). It was interesting to note that fruit beverages produced from fresh red-fleshed plums are currently not readily available in South Africa. The total production of plums in South Africa has shown a steady increase from 32911 tons in 2000 to 626574 tons in 2008. Only a small percentage (1.9%) of the plums produced in South Africa, however, undergo processing (Deciduous Fruit Producers' Trust, 2009).

Plums are known to not only contain various sugars, acids, pectins, tannins and enzymes, but also polyphenolic compounds (Walkowiak-Tomczak *et al*., 2008). A high correlation is generally observed between polyphenolic content and antioxidant activity of plant extracts (Kahkonen *et al*., 1999). Fortunately, compounds other than polyphenolic compounds, such as ascorbic acid, may contribute to the total antioxidant activity of plums (Walkowiak-Tomczak *et al*., 2008).

Plum skin is considered a greater source of polyphenolic compounds than plum flesh (Nunes *et al*., 2008). Plum skin is generally not used during production of a plum juice or nectar, but polyphenolic compounds from plum skins can be recovered by extraction for addition to plum beverages. In this way, enhanced polyphenolic content can be achieved. It is, however, known that polyphenolic compounds can contribute to the development of astringency, an important mouthfeel attribute, which can easily become disadvantageous (Robards *et al*., 1999). Plums also have a relatively high organic acid content (Gil *et al*., 2002). Consumer acceptance of the plum beverages might therefore not only be influenced by possible high levels of acidity, but also by possible high levels of astringency caused by plum skin extract addition (Brossaud *et al*., 2001).

In addition, it is important to study the factors that influence antioxidant activity and the stability thereof in fruit beverages. The factors include temperature, pH, total soluble solids content, ascorbic acid content, storage conditions, atmospheric oxygen (Duda-Chodak & Tarko, 2007), exposure to ultraviolet light, enzymatic degradation (Kalt, 2005), and physical operations, such as slicing and peeling (Piga *et al*., 2003). In the case of plums, variations in polyphenolic composition can result from genetic and environmental factors (Robards *et al*., 1999). Plums should be eating-ripe before processing (Díaz-Mula *et al*., 2008), as ripening results in an increase in anthocyanin content (Usenik *et al*., 2009).

In view of the above, there is a definite demand for the development of a functional plum beverage with a high polyphenolic content, however, from a compositional and production point of view there are several research challenges. This project will focus on some of these challenges.

RESEARCH AIMS

The aims of this research project include the following:

- Development of a red-fleshed functional plum beverage with a high total polyphenolic content.
- Characterisation of the red-fleshed functional plum beverage in terms of colour, polyphenolic content, and antioxidant activity.
- Benchmarking of the red-fleshed functional plum beverage against similar commercial beverages.
- Determination of the sensory profile and consumer acceptance of the red-fleshed functional plum beverage.
- Determination of the shelf life stability of the red-fleshed plum beverage in terms of sensory profile, colour, polyphenolic content and antioxidant capacity.

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Chapter 2
Literature review

INTRODUCTION

The term “functional foods”, first introduced in Japan in the 1980’s, is a concept that encompasses the health benefits drawn from certain food products (Hasler, 1998). Loosely defined as foods or dietary compounds that could contribute health benefits beyond that of basic nutrition (Hasler, 1998), many functional foods are rich in polyphenolic compounds that have been found to relieve or eliminate oxidative stresses that cause damage to macromolecules, such as DNA, protein and lipids (Ames *et al*., 1993). Specifically, polyphenolic compounds in plums are considered to possess many health-promoting properties, including the ability to reduce the risk of heart disease and promote improved bone growth (Chong *et al*., 2010). The major components found in plums include the anthocyanins, cyanidin-3-glucoside and cyanidin-3-rutinoside, the flavonols, quercetin-3-glucoside, quercetin-3-rutinoside and quercetin-3-xyloside, and the hydroxycinnamic acids, chlorogenic and neochlorogenic acid (Nunes *et al*., 2008).

In this literature review, the following will be discussed: Background information on functional foods and the polyphenolic compounds found in functional foods, as well as the benefits and problems surrounding functional foods; the potential of plums as a functional food ingredient and the various polyphenolic compounds found in plums, as well as the polyphenolic and antioxidant compounds found in different fruits; specifications regarding the development of a functional beverage, and methods of sample and skin extract preparation; different methods of analyses used to measure the antioxidant activity and total polyphenolic content of high antioxidant foods; general information regarding sensory analysis and consumer analysis, along with sensory methods conducted on similar products; factors regarding the stability of polyphenolic compounds and methods of stability testing; and the influence of ascorbic acid on anthocyanins during storage.

FUNCTIONAL FOODS

Background

The scientific definition of functional foods is commonly considered as the “foods or dietary components that may provide a health benefit beyond that of basic nutrition” (Hasler, 1998). The term “functional food” was first introduced in Japan in the late 1980’s, but the concept, however, only took flight in the early 1990’s (Hasler, 1998). This ran counter to trends involving the removal and reduction of food components, such as fat, sugar and salt, which often have negative health connotations (Wrick, 2003). Japan is currently the only country in the world that has legally defined functional foods (Arai, 1996). It is also the only country in the world that has an institution that deals

specifically with the implementation of functional food labelling and health claim regulations. Foods that are deemed adequate by regulating institutions, bear a seal of approval to indicate that claims made on the product are legitimate. Products bearing this seal can be identified as a "food for specified health use" (FOSHU) (Hasler, 1998).

The Japanese Ministry of Health states that FOSHU refers to 'foods containing ingredients with functions for health and officially approved to claim its physiological effects on the human body' (Ministerial Ordinance, 1952; Ministerial Ordinance, 1991). It also states that these foods should be consumed for the maintenance or promotion of health and can be used by consumers who are trying to control a certain health condition, such as hypertension. A FOSHU approved product is required to be free of excess salt, fat and sugar and proof must be given that the beneficial components of the product still comply with the product specifications by the time of consumption. Other requirements for FOSHU approval include documentation of quality control methods, a list of ingredients, processing procedures, product specifications, and the methods of chemical and physical analyses conducted on the product (Ministerial Ordinance, 1952; Ministerial Ordinance, 1991).

In the United States of America (USA), there is a conservative trend in relaying health claims on products (Wrick, 2003). This has been attributed to the "industry's history of conflict-avoidance" with the Food and Drug Administration (Wrick, 2003). There is currently no legal definition for functional foods or a governmental institution that regulates functional foods in the USA (Anon., 2009a). Similarly, South Africa currently has no legal definition or specific governmental institution responsible for regulating functional foods. The current food labelling regulations, however, state that subject to the provisions of the Medicines and Related Substances Control Act (1965), the word "cure" or other medical claims, including prophylactic and therapeutic claims, is prohibited (Department of Health, 2010). Claims for antioxidants as nutrients must be subjected to regulation of the Medicines and Related Substances Control Act (Department of Health, 1965) and undergo pre-market approval and registration by the South African Health Products Regulatory Authority (Department of Health, 2010).

Polyphenolic compounds in functional foods

Many functional foods, especially fruits, are rich in polyphenolic compounds (González-Molina *et al*., 2009). These compounds are the most prevalent phytochemicals in most fruits and are considered potent in vitro antioxidants (Moyer *et al*., 2002). Polyphenolic compounds found in nature (Figure 1) can be classified into the following groups: phenolic acids, flavonoids, stilbenes, coumarins, and tannins. Phenolic acids are sub-divided into hydroxycinnamic and hydroxybenzoic acids, while the flavonoids consist of flavonols, flavones, isoflavones, flavanones, flavan-3-ols, anthocyanidins, and isoflavones. Approximately 5000 different polyphenolic compounds have been discovered in nature, of which 2000 are flavonoids (Wrolstad, 2004). Of the 2000 flavonoids, 600 have been identified as anthocyanins (Wrolstad, 2004).

Benefits of functional foods

Polyphenolic and antioxidant compounds are hypothesised to be responsible for many health benefits (Wrolstad, 2004). Polyphenolic compounds are secondary metabolites known for their ability to directly trap or scavenge free radicals through reactions with antioxidant enzymes (Medina *et al*., 2007). These compounds have anti-inflammatory (Carlo *et al*., 1999), hepatoprotective (Hemingway & Larks, 1988), and anticancer (Thomasset *et al*., 2006) activities and reducing the risk of cardiovascular (Chong *et al*., 2010) and neurodegenerative diseases (Aquilano *et al*., 2008), immune-system decline, brain dysfunction, and cataracts (Kehrer & Smith, 1994). Research has shown that these health-benefiting compounds in functional foods work synergistically for added protection from oxidative stress in the human body (Hunter *et al*., 2008).

Oxidative stress results from excess reactive oxygen species, which can damage macromolecules, such as DNA, protein and lipids (Ames *et al*., 1993). Oxidant by-products, such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$), are responsible for oxidative damages. These by-products, which are produced during normal metabolism, are mutagens, which can lead to the accumulation of oxidative damage with age (Ames *et al*., 1993). Functional food products are often associated with good health and longevity, rather than the treatment of a certain disease (Wrick, 2003). This concept has broadened the target market of functional food products, making functional foods marketable to a larger audience (Wrick, 2003). Therefore, the introduction of functional foods containing polyphenolic compounds into the diet is increasingly appealing to aging populations (Hunter *et al*., 2008). A study conducted by Van Kleef *et al*. (2006) concluded that consumers prefer physiology-based, functional health benefits, such as the prevention of cardiovascular diseases and osteoporosis, to the psychology- or behavioural-based benefits, which include stress relief, cosmetic improvements, and increased energy.

Functional foods are not restricted to foods containing polyphenolic compounds. Salmon contains omega-3 fatty acids, which aid in lowering blood cholesterol and stimulating brain function (Sun *et al*., 2006), while certain margarines contain mono-unsaturated fatty acids, which assist in maintaining blood pressure and decreasing cardiovascular disease risks (Grey, 2002). Olive oil is primarily composed of the beneficial fatty acid, oleic acid, but does contain polyphenolic compounds, such as oleuropein (Stark & Madar, 2002). Olive oil also contains tocopherols, carotenoids, and phytosterols, which decrease the risk of cardiovascular disease, breast cancer and hypertension, while enhancing immune response (Stark & Madar, 2002). Other functional foods include soy, oatmeal, flaxseed, tomatoes, and garlic (Hasler, 1998).

Problems surrounding functional foods

Functional foods are often confused with nutraceuticals and novel foods (Arvanitoyannis & Van Houwelingen-Koukaliaroglou, 2005). Novel foods are foods that are new, non-traditional, and different when compared to the foods currently available on the market (Food Standards Australia New Zealand, 2010). Novel foods have no history of safe use (Food Standards Australia New

Zealand, 2010). Nutraceuticals are herbal remedies, often sold in pill or tablet form (Linus Pauling Institute, 2010). Unlike nutraceuticals, functional foods must overcome the sensory hurdle of potential inherently unpleasant taste and mouthfeel characteristics generally associated with the phytochemicals that provide health benefits to foods (Wrick, 2003). Astringency and bitterness are unpleasant mouthfeel and taste characteristics, respectively, which can occur in some functional food products (Brossaud *et al*., 2001). Bitterness and astringency both result from polyphenolic compounds. The molecular sizes of these polyphenolic compounds influence the level of bitterness and astringency experienced. Flavan-3-ol monomers are considered more bitter than astringent (Brossaud *et al*., 2001). Increased astringency results from increased concentrations of oligomeric and polymeric flavan-3-ols (Lea & Timberlake, 1974). Hydroxycinnamic acids (Hufnagel & Hofmann, 2008) and anthocyanins (Brossaud *et al*., 2001) can also contribute to astringency.

Finding the optimum balance between phytochemical concentration and sensory characteristics is time-consuming and complicated (Wrick, 2003). Attempts can be made by producers to mask unpleasant sensory attributes, causing production delays and increased costs (Wrick, 2003). Hunter *et al*. (2008) suggested that a structured approach be taken when screening ingredients during the development of a functional food product, while Van Kleef *et al*. (2006) suggested that adequate consumer research be considered in order to distinguish a successful product from unsuccessful products. These factors make the development of a functional product complex, expensive, and risky (Van Kleef *et al*., 2006).

Scepticism surrounds functional foods and the health claims of these foods, as control over claims is sparse (Arvanitoyannis & Van Houwelingen-Koukaliaroglou, 2005). Fears of possible false or exaggerated claims surrounding functional foods spawn from a lack of convincing scientific evidence or controlling bodies in many countries (Arvanitoyannis & Van Houwelingen-Koukaliaroglou, 2005). This causes a large majority of functional food products to fail when introduced into the market place (Van Kleef *et al*., 2006). Inadequate positioning within the market place is also believed to cause functional foods to fail (Wrick, 2003). This can be rectified with proper identification of target consumers, which could, unfortunately, prove to be complicated and expensive, as a potential product market is governed by knowledge of the consumer group and socio-graphic segmentation (Wrick, 2003).

PLUMS AS FUNCTIONAL FOOD INGREDIENT

Taxonomically, plums are placed in the family *Rosaceae* of the genus *Prunus* (Potter *et al*., 2007). This genus also contains other stone fruits such as peaches, cherries, and apricots (Potter *et al*., 2007). Plums are broadly grouped into two distinct species, namely the European plums (*Prunus domestica* L.) and the Japanese plums (*Prunus salicina* L.). European plums are usually smaller than Japanese plums and are more often used in prune production, while Japanese plums, are mostly destined for the fresh fruit market.

Plums are known to contain large amounts of polyphenolic compounds, which are non-toxic and non-mutagenic (Bridle & Timberlake, 1996). The polyphenolic compounds in plums bear many health-promoting properties, such as cataract and atherosclerosis prevention or inhibition (Kehrer & Smith, 1994). Heart disease (Chong *et al*., 2010) and degenerative diseases, such as Parkinson's and Alzheimer's diseases (Thomasset *et al*., 2006), are also inhibited by polyphenolic compounds found in plums. The benefits of the flavonols in plums include their antiulcer, antispasmodic, antisecretory, antidiarrhoeal (Carlo *et al*., 1999) and antihepatotoxic properties (Hemingway & Larks, 1988). Reports have also stated that plums can elevate bone formation by increasing serum IGF-I levels (Hooshmand & Arjmandi, 2008). Additionally, plum juice was found by Shukitt-Hale *et al*. (2009) to inhibit age-related cognitive decline in rats.

Polyphenolic compounds in plums

Plums contain a variety of polyphenolic compounds, including hydroxycinnamic acids (Donovan *et al*., 1998), anthocyanins (Clifford, 2000), flavonols (Tomás-Barberán *et al*., 2001) and flavan-3-ols (Robards *et al*., 1999). Polyphenolic compounds in fruits are generally located in greater concentrations in the skins than in the flesh (Nunes *et al*., 2008). Although polyphenolic compounds are common in the human diet (Manach *et al*., 2004), many of these compounds are often poorly absorbed from the intestine. Polyphenolic esters, glycosides, and polymers cannot be absorbed by the small intestine and must be hydrolysed before absorption (Manach *et al*., 2004).

Phenolic acids

Hydroxycinnamic acids are some of the most prevalent polyphenolic compounds in nature (Medina *et al*., 2007). These compounds are rarely found in free form in nature and tend to bind to form glycosylated, quinic acid, shikimic acid, or tartaric acid derivatives (Manach *et al*., 2004). Hydroxycinnamic acids (Figure 1), the derivatives of cinnamic acid, make up one of two classes of phenolic acids, the other being derivatives of hydroxybenzoic acid (Lafay & Gil-Izquierdo, 2008). Hydroxybenzoic acids are generally only found in low concentrations in fruits and vegetables and do not occur in plums (Lafay & Gil-Izquierdo, 2008). Caffeic acid accounts for approximately 70% of total hydroxycinnamic acids in fruits (Macheix *et al*., 1990). The most prevalent hydroxycinnamic acid is chlorogenic acid, consisting of caffeic acid esterified to quinic acid (Manach *et al*., 2004).

Hydroxycinnamic acids consist of benzene as a basis bound to a propenoic acid (Lafay & Gil-Izquierdo, 2008). Hydroxycinnamic acids generally differ in hydroxyl group numbers, as well as their reducing capacities (Manach *et al*., 2004). Caffeic acid donates a large amount of electrons, followed by ferulic acid and chlorogenic acid (Manach *et al*., 2004). Reports indicate that the absolute antioxidant capacity of a hydroxycinnamic acid cannot be predicted by the number of hydroxyl groups in the compound, although a higher number of hydroxyl groups generally increases its antioxidant capacity (Medina *et al*., 2007).

Some of the most predominant compounds in plum flesh are hydroxycinnamic acid derivatives (Nunes *et al*., 2008). The main hydroxycinnamic acids in plum flesh are chlorogenic acid and neochlorogenic acid, structural isomer of chlorogenic acid. Neochlorogenic acid accounts for 67% to 88% of the total hydroxycinnamic acids in plum flesh. The remaining hydroxycinnamic acid content is composed of less prevalent compounds, such as 3-*O*-caffeoylshikimic acid and 3-*O*-feruloylquinic acid. Similar results were found for plum skins (Nunes *et al*., 2008).

Hydroxycinnamic acids are rapidly absorbed from the stomach or the small intestine when ingested in free form (Lafay & Gil-Izquierdo, 2008), while hydroxycinnamic acid derivatives are usually hydrolysed in the upper part of the gut (Manach *et al*., 2004). As in chlorogenic acid, the esterification of caffeic acid with quinic acid dramatically decreased its absorption compared to caffeic acid (Lafay & Gil-Izquierdo, 2008). Lafay *et al*. (2006) determined that merely 8% of chlorogenic acid was absorbed in the small intestine, which was 2.4 times lower than the absorption of caffeic acid (Lafay *et al*., 2006).

Flavonoids

Flavonoids found in plums broadly include flavonols, flavan-3-ols and anthocyanins (Figure 2). Flavonoids are diphenylpropanes that share a common structure (Cao *et al*., 1997). The structure of flavonoids consists of two aromatic rings bound together by three carbon atoms that form an oxygenated heterocycle (Manach *et al*., 2004).

Flavonols possess a C-ring structure with a double bond at the 2-3 position (Hollman & Arts, 2000). Flavonols are predominantly found in the glycosylated form with sugar moieties, such as glucose, galactose, arabinose, xylose, glucuronic acid, and rhamnose (Manach *et al*., 2004). Flavonols usually occur in plants as *O*-glycosides, but may also rarely occur as *C*-glycosides (Hollman & Arts, 2000).

The major flavonols found in plums include quercetin-glycosides and kaempferol-glycosides (Manach *et al*., 2004). Rutin, the rhamnoglucoside of quercetin, was identified as the principal flavonol glycoside in plums (Nunes *et al*., 2008). Other quercetin glycosides in plums include quercetin-3-glucoside, quercetin-3-rhamnoside, and quercetin-3-xyloside. In small amounts, kaempferol-3-rutinoside, kaempferol-3-glucoside, kaempferol-3-galactoside, and kaempferol-3-arabinoside-7-rhamnoside have also been identified in plums (Nunes *et al*., 2008). Manach *et al*. (2004) found flavonols to be prevalent in the skins of fruits and the leaves of plants. This was attributed to the biosynthesis of these compounds, which are stimulated by light. This hypothesis was supported by differences between the concentrations of flavonols in fruits of the same tree and on different sides of the same fruit, depending on exposure to light (Manach *et al*., 2004).

Sugar moieties from flavonol conjugates can be hydrolysed by enzymes, which are produced by colonic bacteria, resulting in flavonol aglycones (Hollman & Arts, 2000). These bacteria also degrade the flavonol aglycones. The bacterial degradation involves the splitting of the heterocyclic oxygen-containing ring. The ensuing degradation products are absorbed. The

bioavailability of quercetin-3-glucoside was, however, determined as superior to that of other various quercetin glycosides, including quercetin-3-rutinoside (Hollman & Arts, 2000). Quercetin glucosides are efficiently absorbed in the small intestine via a sodium-dependent glucose transporter, SGLT1 (Manach *et al*., 2004). The glucosides are hydrolysed by cytosolic glucosidase for penetration through the cells. An alternate pathway involves lactase phloridzine hydrolase (Manach *et al*., 2004).

Flavan-3-ols are flavonoid compounds that are found in certain fruits and vegetables (Medina *et al*., 2007), as well as in teas, wines and legumes (Hollman & Arts, 2000). Flavan-3-ols are hypothesised to scavenge free oxygen radicals, in addition to chelating metal ions, specifically iron (Medina *et al*., 2007). Flavan-3-ols generally differ with respect to the presence of the pyrogallol moiety and the galloylated residues. Reports indicate that the gallate esters of (+)-catechin, such as (+)-catechin gallate and (+)-gallocatechin gallate, are able to donate more electrons than (+)-catechin and (+)-gallocatechin. This is due to the pyrogallol moiety, which provides more electrons than the catechol group. Flavan-3-ols differ in their abilities to chelate metal ions due to the number of *o*-hydroxilic groups in the compound. This explains why (+)-catechin gallate and (+)-gallocatechin gallate are more effective chelating agents than (+)-catechin and (+)-gallocatechin (Medina *et al*., 2007).

Flavan-3-ols are some of the principal compounds found in plum flesh (Medina *et al*., 2007). Nunes *et al*. (2008) have reported the occurrence of flavan-3-ols, such as procyanidin monomers, A and B dimers, and trimers in plums. Flavan-3-ols have been reported to make up 4 to 8% of total polyphenolic content of plums (Donovan *et al*., 1998). Flavan-3-ols, such as (+)-catechin, made up 27% to 85% of the total polyphenolic content in the skins of certain plums (Nunes *et al*., 2008). Procyanidins, such as procyanidin B1, were found in higher concentration in the flesh of the plums than in the skins. Plum flesh contains small amounts of (-)-epicatechin. Less frequently identified flavan-3-ols in plums include procyanidin B7 and A-type procyanidin dimers (Nunes *et al*., 2008).

Flavan-3-ols are often present in the aglycone form as monomers, oligomers, or esters (Hackman *et al*., 2008). During digestion and transfer across the small intestine, in addition to transport in the liver, flavan-3-ols are rapidly metabolised. Flavan-3-ols, which are not absorbed in the small intestine, are generally metabolised by bacteria in the colon. The metabolites produced are rapidly excreted in the bile and urine. Flavan-3-ols and procyanidins are somewhat stable in stomach acid. Most procyanidins are degraded to monomers or dimers prior to absorption (Hackman *et al*., 2008). Proanthocyanidins possess limited absorption capacities through the small intestine due to their complex polymeric structure and large molecular weight (Manach *et al*., 2004). Procyanidin B2 and B3 have been found to have little and no absorptive capacities, respectively (Manach *et al*., 2004).

Anthocyanins are the natural blue, violet, or red pigments that occur in fruits, vegetables and flowers (Wang *et al*., 1996). These compounds are mostly found in the epidermal tissue of

blue, violet, or red fruits, serving to protect the fruit from ultraviolet radiation and acting as an antimicrobial barrier (Wrolstad, 2004).

The hue and structure of anthocyanins depends on pH and the presence of copigments (Clifford, 2000). Anthocyanins occur in the red flavylium cation form at low pH (Clifford, 2000). Bridle and Timberlake (1996) stated that anthocyanins have very little colour above pH 3.5 and that these compounds have an increase in colour intensity when associated with copigments that are found in the native environment of these anthocyanins. Anthocyanins are glycosides of anthocyanidins (Clifford, 2000). Anthocyanins broadly vary with respect to the number and position of hydroxyl and methoxyl substituents of the basic anthocyanidin skeleton, the type, number, and positions of the sugars that are bound to the skeleton, and the extent of the acylation of these sugars. The sugars that are regularly found attached to the anthocyanidin skeleton of the anthocyanins include glucose, galactose, rhamnose, and arabinose. Other sugars found in anthocyanin structures include rutinoside, sophorosides and sambubiosides. The anthocyanins also vary with respect to the sugar acylating agent, which could include cinnamic acids, such as caffeic acid, *p*-coumaric acid, ferulic acid and sinapic acid (Clifford, 2000).

Anthocyanins account for 4 to 9% of total polyphenolic content in fresh plums (Donovan *et al*., 1998). The anthocyanins found in plums include cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-galactoside, and cyanidin-3-acetyl-glucoside, as well as peonidin-3-glucoside and peonidin-3-rutinoside (Usenik *et al*., 2009). The same types of anthocyanins are generally found among most plum cultivars. Variations in anthocyanin content have, however, been reported between cultivars and fruit samples of the same cultivar (Usenik *et al*., 2009). Anthocyanins also vary considerably with environmental influences and growing location (Moyer *et al*., 2002). Interestingly, the most common anthocyanin found in nature is cyanidin-3-glucoside (Manach *et al*., 2004).

Humans are considered as being well adjusted to anthocyanin ingestion (Bridle & Timberlake, 1996). In 1971, the average daily intake of anthocyanins in USA was estimated to be approximately 215 mg/day during summer and 180 mg/day during winter. Similar results were found for the consumption of anthocyanins in Italy in 1996 (Bridle & Timberlake, 1996). A study conducted by Lapidot *et al*. (1998), unfortunately, showed that only 1.5 to 5.1% of anthocyanins are absorbed. Similarly, Talavéra *et al*. (2006) found that anthocyanins possess limited bioavailability.

Polyphenolic and antioxidant compounds in different functional fruits

Apart from plums, certain other fruits, such as nectarines and peaches, also contain large amounts of polyphenolic compounds (Manach *et al*., 2004). The same is true for fruits, such as blueberries, raspberries and strawberries (Kalt *et al*., 1999). These berries are considered especially high in anthocyanins (Kalt *et al*., 1999).

Donovan *et al*. (1998) has determined that blueberries (4500 mg of phenolics/kg fruit) possess a higher total polyphenolic content than both cherries (850 mg of phenolics/kg fruit) and plums (1107 mg of phenolics/kg fruit), while Red Flame seedless table grapes (<250 mg of phenolics/kg of grape) seem to contain a lower total polyphenolic content than cherries. Grape juice contains tartaric acid esters of hydroxycinnamates and proanthocyanidins (Donovan *et al*., 1998). Pinot noir grapes only contain anthocyanin-3-glucosides (Cheynier, 2005), while most other red grape cultivars contain both anthocyanin-3-glucosides and acylated anthocyanins (Cheynier, 2005).

Flavonols have been found in foods, such as fruit, vegetables, cereals, legumes, teas, wines (Manach *et al*., 2004). Flavanones are generally only found in citrus fruits, while isoflavones are restricted to legumes. Flavonols, such as quercetin glycosides have also been found in the skins of red apples, together with anthocyanins, such as such as cyanidin-3-galactoside, hydroxycinnamic acids, such as chlorogenic acid, and flavan-3-ols, such as (-)-epicatechin and procyanidin B2 (Manach *et al*., 2004). Flavan-3-ols have been noted in pear juice (Donovan *et al*., 1998). Anthocyanins, such as punicalagin, can be found in pomegranates (Tezcan *et al*., 2009).

Many red, blue and violet fruit beverages are available on the market. These include beverages prepared from high antioxidant-containing fruits, such as blueberries, raspberries and strawberries (Table 1). Plums generally possess a low anthocyanin content compared to fruits such as blackberries and blueberries (Table 1). Plums possess an anthocyanin content similar to strawberries and a flavonol content similar to strawberries, raspberries, and blackberries, but a greater proanthocyanidin content than blackberries, blueberries, red grapes, raspberries, and strawberries. The flavan-3-ol content of the plums was also found to be on par with blueberries and red grapes (Table 1).

Table 1. Ranges of total anthocyanin, flavonol, proanthocyanidin, and flavan-3-ol contents of red, blue and violet fruits (mg/100 g) (compiled by Linus Pauling Institute, 2010[a])

Anthocyanin-rich fruits	Anthocyanins	Flavonols	Proanthocyanidins	Flavan-3-ols
Blackberry	89-211	0-2	6-47	13-19
Blueberry	67-183	2-16	88-261	1
Grapes, red	25-92	3-4	44-76	2
Raspberries, red	10-84	1	5-59	9
Strawberry	15-75	1-4	97-183	ND
Plum	2-25	1-2	106-334	1-6

[a] compiled from Henning *et al*., 2003; Moyer *et al*., 2002; Ryan and Revilla, 2003; U.S. Department of Agriculture, 2003; U.S. Department of Agriculture, 2004; Vrhovsek *et al*., 2004; ND - not determined.

PRODUCT DEVELOPMENT

Regulations governing the development of fruit-based beverages

The regulations governing fruit beverages in South Africa recognises seven different classes for fruit beverages (Department of Agriculture and Fisheries, 1980), including fresh juice, unsweetened juice, sweetened juice, nectar, squash, drink and imitation drink. A range of fruits are specified in the South African regulations, while others, including plums are considered unspecified fruits and denoted with an 'X' (Department of Agriculture and Fisheries, 1980). Regulations with regard to beverages from unspecified fruits will be discussed further.

All beverages are stated by the regulations to be prepared from fruit of good quality (Department of Agriculture and Fisheries, 1980). All beverages should not contain any additives, should not be subjected to any preserving processes other than chilling, should be clean and free from foreign matter, and should be practically free from seeds, bits of seeds, or bits of peel. The unsweetened juice and the sweetened juice should be free from deterioration or spoilage and should have the characteristic flavour and colour of the kind of natural juice concerned. These juices should be effectively treated against deterioration and spoilage by means of any permitted method. If packed under a vacuum, the juice shall have a minimum vacuum of 17 kPa and the juice shall be free from spoilage in excess of 0.25% of the containers in the consignment (Department of Agriculture and Fisheries, 1980).

A fresh juice is stated in the South African regulations to consist of natural juice, intended for consumption within 2 h of extraction thereof (Department of Agriculture and Fisheries, 1980). An unsweetened juice should contain no additives other than the permitted preservatives and natural fruit essence of the fruit concerned, ascorbic acid and carbon dioxide. In ready-to-drink form, an unsweetened juice must have a minimum natural juice content of 100% and a °Brix-value of not less than 12 °Brix. A sweetened juice should contain no additives other than permitted natural sweeteners not exceeding 5% (m/m), other permitted sweeteners, water, natural fruit essence of the fruit species concerned, ascorbic acid and carbon dioxide, and permitted preservatives. In ready-to-drink form, a sweetened juice should have a minimum natural juice content at standard strength of 90% (v/v) and a °Brix value of no less than 12 °Brix (Department of Agriculture and Fisheries, 1980).

A nectar consists of the unspecified juice which complies with the requirements of the unsweetened juice or sweetened juice sub-regulations (Department of Agriculture and Fisheries, 1980). By virtue of the addition of water or permitted substances, the nectar contains less than 90% (v/v) unspecified juice in the ready-to-drink form. A nectar should have a minimum of 40% fruit juice (v/v) in the ready-to-drink form, with a minimum total soluble solids content of 12 °Brix (Department of Agriculture and Fisheries, 1980).

The regulations governing fruit beverages in the United Kingdom state that fruit juice is juice prepared directly from the fruit and should not be concentrated or reconstituted from

concentrated juice (Food Standards Agency, 2003). Fruit nectars are defined as a product that is made by the combination of fruit juice, fruit juice from concentrate, concentrated fruit juice, dehydrated fruit juice, fruit puree, or a mixture of these products with water and added sugar and/or honey and/or sweeteners. The regulations require minimum quantities of fruit juice, fruit puree, or a mixture of such juices and purees for these products, depending on the type of fruit used (Food Standards Agency, 2003).

Sample preparation processes

Limited information regarding the processing of plum nectars or juices is currently available. Will and Dietrich (2006) prepared plum juice by heating the pitted plums to 90°C in a tube exchanger and placing the fruit in a mash buffer tank for 20 min. This treatment served as the minimum requirement for releasing anthocyanins from the skins into the liquid phase to yield an intensely coloured juice. Thereafter, the mash was placed in a second heat exchanger to be cooled to 50°C. After cooling, the mash was pumped into a temperature-controlled stirring tank to be stirred for 60 min. The stirring would allow for an adequate decrease in viscosity. Pectin lyase and ascorbic acid was subsequently added to the mash for prevention of possible oxidation. The mash was then sent through the final decanter extraction where the juice was separated from the fruit solids. Thereafter, the juice was hot-filled (85°C) into glass bottles and left to cool. The addition of demineralised water and sucrose to the juice created a nectar.

Similarly, Chang *et al*. (1994) prepared a juice by crushing plums and adding 0.2% Clarex® L (Solvay Enzymes Inc., Elkhart, USA), which aided juice extraction. The macerate was held at 49°C for 3 h before undergoing pressing. Sodium bentonite (5.0%) and gelatine (1%) solutions at 0.05% (w/w) juice were added to the juice, which was obtained from pressing, to facilitate clarification. The mixture was left overnight (2 - 3°C) before undergoing racking and filtration. Subsequently, the juice was subjected to high temperature short time pasteurisation (85°C for 90 s) and was frozen until further analyses could be performed.

A clear strawberry juice was prepared by Oszmiański and Wojdyło (2009) by pressing the pulp, using a Zodiak laboratory hydraulic press and press cloth. The extracted juice was heated in a microwave oven for 5 min until the product had reached an internal temperature of 90°C. Thereafter, the juice was cooled to 45°C and treated with pectinase. The mixture was stirred for 30 min at 40°C before undergoing a clarification process using gelatine, baykisol 30, and bentonite G. This was followed by centrifugation and pasteurisation.

Limited information regarding skin extraction methods is available in literature. Polyphenolic compounds have been extracted from grape skins using an ultrasonic bath (Corrales *et al*., 2009). The ultrasonic bath was set at a frequency of 35 kHz for 30 min. The skins were transferred to a water bath at a temperature of 70°C for 2.5 h, increasing the solid/liquid ratio to 1:20. Alternatively, Costoya *et al.* (2010) prepared a skin extract from grape skins by using a selection of enzymes. During their study, the pomace samples were pressed and subjected to extraction in a rotary

shaker at a constant stirring rate (140 rpm). The extraction was conducted at 50℃ for 30 min at a solvent/solid ratio of 1:1. The enzymes were added with water, making the incubation time of sample similar to the extraction time. The enzyme/substrate ratio was 15 g/kg.

POLYPHENOLIC AND ANTIOXIDANT ANALYSES

Polyphenolic analysis

Spectrophotometric and HPLC methods are generally used to quantify polyphenolic compounds. The total polyphenolic content of foods and beverages is predominantly measured using the Folin-Ciocalteau method (Singleton & Rossi, 1965). The Folin-Ciocalteau method is easy, reproducible and accurate (Prior *et al*., 2005). It involves the oxidation of polyphenolic compounds by a molybdotungstophosphoric heteropolyanion reagent to yield molybdotungstophosphate blue, which is spectrophotometrically measured (Singleton & Rossi, 1965).

The total anthocyanin content of foods and beverages can be measured spectrophotometrically using a method first described by Ribéreau-Gayon and Stonestreet (1966). The method involves the addition of an acidic reagent to the sample. At the acidic pH of the mixture, anthocyanins are all in the red flavylium ion form, which can be spectrophotometrically measured at 520 nm. Corrections for turbidity can be made by subtracting the absorbance at 700 nm. This method is highly reproducible, but unfortunately only renders approximate results (Ribéreau-Gayon and Stonestreet, 1966).

Polyphenolic compounds in plums can be quantified using HPLC with a diode array or mass spectrometric detector (Gil *et al*., 2002). Electrospray mass spectroscopy and tandem mass spectroscopy can be used to obtain structural information on individual compounds (Nunes *et al*., 2008). Reversed-phase HPLC is generally used to quantify polyphenolic compounds in plums. A high acid content is required in the mobile phases for good separation of the anthocyanin compounds to be obtained (Nunes *et al*., 2008).

Antioxidant analyses

Many methods are available for determining antioxidant activity (Prior *et al*., 2005). The 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium radical cation ($ABTS^{\bullet+}$) scavenging assay is based on the ability of the antioxidants to scavenge a stable synthetic radical cation (Bartosz *et al.,* 1998). The total radical-trapping antioxidant parameter (TRAP) assay monitors the interference of antioxidant compounds with the reaction between 2,2'-azo-bis-(2-amidinopropane) dihydrochloride (AAPH)-generated peroxyl radicals and a target probe (Wayner *et al*., 1986). The total oxidant scavenging capacity (TOSC) assay allows for the quantification of the absorbance capacities of antioxidants specifically toward peroxynitrite, and hydroxyl and peroxyl radicals (Winston *et al*., 1998). Chemiluminescence assays are based on the oxidant reactions with markers to produce species that emit chemically induced light (Whitehead *et al*., 1992). The

photochemiluminescence system, involves the photochemical generation of superoxide radicals by optical excitation a photosensitizer combined with a chemiluminescent detection (Popov & Lewin, 1999). Croton bleaching measures the autooxidation of carotenoids, induced by light or heat, which bleaches the carotenoids, degrading the antioxidants that donate hydrogen atoms to quench radicals (Burda & Oleszek, 2001). The low-density lipoprotein (LDL) oxidation assay measures antioxidant status. The copper reduction assay is based on the reduction of Cu(II) to Cu(I) through combined actions of the antioxidants in a sample (Prior *et al*., 2005).

The popular oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant potential (FRAP), and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH$^{\bullet}$) scavenging assays will be discussed in more detail. The ORAC method (Huang *et al*., 2002) is especially popular in the USA. In this assay, AAPH acts as a peroxyl radical generator that reacts with a fluorescence indicator, fluorescein, which decreases the fluorescence measured (Huang *et al*., 2002). The ORAC method can detect both hydrophilic and hydrophobic antioxidants (Prior *et al*., 2005). The ORAC method is a readily automated method that provides an accurate representation of how antioxidants react with lipids *in vitro*. Unfortunately, this method is very temperature sensitive, making the reproducibility of the assay difficult. It also requires long analysis times (Prior *et al*., 2005).

The FRAP assay is a fast, easy to employ and inexpensive means of measuring antioxidant activity (Prior *et al*., 2005). The FRAP assay does not require any specialised equipment and can be implemented using automated, semi-automated, or manual methods. The FRAP method spectrophotometrically determines the ferric reducing abilities of antioxidants in the samples by measuring the reduction of 2,4,6-tripyridyl-*s*-triazine (TPTZ)-Fe^{3+} to TPTZ-Fe^{2+} (Prior *et al*., 2005). Unfortunately, results obtained from this method are not always consistent and depend on the duration of the analysis. The duration times of the analysis depend on the type of compound being measured. A single-point absorption endpoint does not necessarily represent the complete reaction of the sample (Prior *et al*., 2005).

The DPPH$^{\bullet}$ radical scavenging assay is often used to determine the free radical scavenging capacity of antioxidants in a sample as it is fast and reproducible (Bermúdez-Soto & Tomás-Barberán, 2004). The assay determines the potential of antioxidants to donate hydrogen to DPPH$^{\bullet}$, which is associated with a decrease in colour (Ndhlala *et al*., 2008). The interpretation of results can be complicated when the test compounds have a spectrum that overlaps with that of DPPH$^{\bullet}$ (Prior *et al*., 2005). In such a case, a sample blank is prepared and analysed. If the reaction time is not long enough, the slow reactivity of DPPH$^{\bullet}$ with some antioxidants can cause an underestimation of activity (Prior *et al*., 2005).

SENSORY AND CONSUMER ANALYSES

Sensory analysis

Descriptive sensory analysis is conducted to compare products with each other or to identify the sensory characteristics of a specific product (Lawless & Heymann, 1998). Sensory analysis is often used to determine the acceptability of a newly developed product. A common sensory technique is quantitative descriptive analysis (QDA), which is often used to describe the sensory characteristics of the samples. These include flavour, mouthfeel, aftertaste and visual aspects of the samples (Lawless & Heymann, 1998).

During QDA, a training phase is implemented to allow panel members to create a scientific language for various product samples (Lawless & Heymann, 1998). This ensures that all the judges use the same, non-redundant terms to adequately communicate with each other. Reference standards are used to teach the judges how to distinguish between terms, such as astringency and bitterness. The training sessions are facilitated by a panel leader who directs the discussion and provides the reference standards and product samples. After consensus has been reached regarding the terms used to describe the product characteristics, a series of trial evaluations are conducted by the judges. The evaluations are conducted in isolated booths where standard sensory practices, such as booth lighting, rinsing between samples, and sample coding are employed. A graphic line scale that is anchored between two fixed verbal endpoints is often used. The order in which the judges rank the intensities of the characteristics of the samples is of greater importance than the part of the scale used, as certain statistical procedures, such as the dependent t-tests, remove the influence of this factor (Lawless & Heymann, 1998).

Results yielded from the evaluations are used to statistically evaluate the performances of an individual judge relative to the whole panel (Lawless & Heymann, 1998). Replications of the sample evaluations should be conducted to determine the consistency of each judge and of the entire panel. The replications allow for the analysis of variance of individual judges across samples to be determined. The number of replications conducted is product and judge dependent. It is used to determine whether discriminations can be made between samples, and is used to determine whether further panel training is required. The statistical analyses conducted on QDA data include analysis of variance (ANOVA), principal component analysis (PCA), factor analysis and cluster analysis. These results are often graphically represented (Lawless & Heymann, 1998).

Consumer analysis

Consumer analysis is conducted towards the end of the product development stages where product formulations have been narrowed down to a manageable subset (Lawless & Heymann, 1998). Consumer analysis aids in determining whether consumers like the product, prefers the product over other products, or finds the sensory characteristics of the product acceptable.

QDA results are often used to interpret the consumer responses to the same samples. These results can give an indication of the possible success of a product with consumers (Lawless & Heymann, 1998). Therefore, results from acceptability studies can be used to determine the best design for the product through combination with product formulation constraints and consumer expectation information (Lawless & Heymann, 1998).

Generally, there are two approaches to consumer analysis; the analysis of preference and the analysis of acceptance (Lawless & Heymann, 1998). Preference analysis involves asking the consumer to choose one sample over another, while acceptance testing requires the consumer to rate the sample independently. Acceptance analysis is often conducted using a nine-point hedonic scale. First invented at the Food Research Division of the Quartermaster Food and Container Institute in Chicago, USA, in the 1940's (Lawless & Heymann, 1998), this scale is based on the assumption that consumer preference exists on a continuum and that preferences can be conveyed through *like* and *dislike* responses. Research conducted revealed that the vertical or horizontal direction and the order in which the responses were given did not affect the results. The nine-point hedonic scale uses an equal-interval spacing method, determined using the Thurstonian method (Lawless & Heymann, 1998), to assign numerical values to consumer response choices using parametric statistical analysis of the results. Furthermore, the nine-point hedonic scale is simple and easy to use. It is reliable, highly response stable, and is independent of regional and panel size. The consumer results obtained from a nine-point hedonic scale can be converted to obtain paired preference or rank data. Results can also be used to conduct preference mapping analyses. Preference mapping is a visualisation method to determine the directions for sample preferences in a spatial model of a product set. This multivariate technique allows for samples to be represented as a space, with samples possessing similar attributes being positioned close to each other. The attributes that differentiate the samples can also be positioned in the space, possibly explaining the differences in liking. Preference mapping projects the market segmentation between different groups of consumers with respect to their product liking. Preference mapping can indicate possible directions for product optimisation (Lawless & Heymann, 1998).

Sensory and consumer analyses performed on fruit beverages

Consumer preference tests have been conducted on products, such as cloudy apple juice, which are similar to the red-fleshed plum nectar formulations (Jaros *et al*., 2009). During a consumer preference analysis conducted by Jaros *et al*. (2009), a group of 110 consumers were asked to evaluate six non-fructose-containing cloudy apple juice samples. The samples were served in 50 mL aliquots in 100 mL glasses at room temperature (21°C). The juice samples were served in a systematically varied order using a latin square design. The consumers were asked to rank the samples from *most preferred* to *least preferred*. No two samples were to be given the same rating. A Friedman test was conducted on the data to determine whether a significant ($P \leq 0.05$) difference existed between the sample preferences. A PCA plot was constructed to visually determine the

intercorrelated variables in the samples. Additionally, ANOVA, multiple comparisons, and cluster analysis were conducted on the consumer results.

A similar consumer analysis was conducted on five blueberry-based drink samples by Endrizzi *et al*. (2009) using 142 consumers. To determine the acceptability of the samples, consumers were asked to rate each sample on a nine-point hedonic scale. The acceptability test included a questionnaire pertaining to the socio-demographics of the consumers, and their knowledge and opinions of antioxidants and fruits. Sample acceptability was analysed using the Friedman test. A multiple comparison test was conducted to determine the existence of homogenous groups within the samples. Consumer segments were determined by their preference for the different samples using clustering around latent variables (CLV). CLV manages the consumer classification in an L-shaped data-structure context (Endrizzi *et al*., 2009).

An in-store consumer test was conducted by Crisosto *et al*. (2004) to determine the degree of liking and acceptance of four ripe Blackamber plum samples with varying total soluble solid content. A nine-point hedonic scale was used in this analysis. A correlation was drawn between the laboratory results of each fruit and the responses of the 100 consumers. The data from the consumer preference test were subjected to ANOVA to determine the least significant difference (LSD) between means (Crisosto *et al*., 2004).

QDA was used by Crisosto *et al*. (2007) to study the sensory qualities of twelve plum and four pluot cultivars. A 10-member trained panel of judges was selected based on their flavour accuracy. The training sessions were conducted at room temperature (21°C) under fluorescent lighting in individual booths. Eight samples were served in a randomised order in soufflé cups and evaluated during each session. The panelists scored the sensory characteristics of the samples on a horizontal line scale anchored by *none* and *more* or *less* and *more*. The correlations between the sensory and chemical attributes of the samples were analysed. ANOVA was conducted to evaluate the significant differences at the 1 and 5% levels. Additional data analyses were conducted to identify particular clusters of cultivars with similar characteristics. The Euclidian distance was calculated using Ward's method as segregation criterion, while a PCA was used to determine the association between the sensory attributes and the cultivars.

STORAGE INFLUENCES ON POLYPHENOLIC COMPOUNDS

Polyphenolic compounds in beverages are influenced by external factors, such as processing and storage conditions (Kalt, 2005). The content of polyphenolic compounds in fruits, such as strawberries and raspberries, decreases with an increase of storage temperature (Kalt, 2005). The anthocyanin levels of the strawberries are said to increase during optimum storage, while the total polyphenolic content and antioxidant capacity of strawberries remain unchanged. The anthocyanin content, total polyphenolic content, and antioxidant capacity of the raspberries increase during optimum storage. Strawberries were shown to present less anthocyanin accumulation in a

controlled atmosphere of high carbon dioxide than in air. This was attributed to the lower activity of the enzymes involved in the synthesis of anthocyanins and the higher pH of the fruit tissue due to the carbon dioxide (Agar *et al.,* 1997). Reports indicate that the polyphenolic content and antioxidant capacity of bananas decrease dramatically during storage, while banana peels presented a definite increase in antioxidant capacity (Kevers *et al*., 2007). A loss in total flavonoids was detected in banana peel and flesh during storage. Apricots were determined by Kevers *et al*. (2007) to decrease in antioxidant capacity during storage at low temperatures.

The antioxidant capacity of polyphenolic compounds in 'Sugar' plums was found to increase significantly ($P \leq 0.05$) with high drying temperatures (60°C), while the opposite was found for 'President' plums (Piga *et al*., 2003). Piga *et al*. (2003) found that when the latter plums were dried at a higher temperature (85°C) the total polyphenolic content was more than double that of the fresh fruits (dry weight basis). This has been attributed to the intermediate oxidation stage of the polyphenolic compounds, portraying a greater antioxidant power than the initial polyphenolic compounds found in the fresh fruit. Higher temperatures are believed to cause a stabilisation step that leads to the formation of new compounds, that may have a higher antioxidant activity than the original polyphenolic compounds present in the fresh plums (Piga *et al*., 2003).

Anthocyanin stability in a model solution during storage has been tested by Cabrita *et al*. (2000). Anthocyanins were stored at 10 and 23°C at different pH levels varying from pH 1 to 12. The anthocyanins showed 70% stability after 60 days of storage at 10°C between pH 1 and 3, with the stability lowering considerably as the pH increased. At 23°C and pH between 1 and 2.4, the anthocyanins presented a lower stability than was observed at the same pH values at 10°C. The presence of light and certain degrading enzymes or complexing compounds reportedly degrades anthocyanins (Will & Dietrich, 2006). Transition metals, such as copper ions, act as catalysts that produce hydrogen peroxide, assisting in the degradation of anthocyanins (García-Viguera & Bridle, 1999).

A storage experiment was conducted by Will and Dietrich (2006) with colour- and cloud-stable plum juice formulations. The juice samples were stored in a dark, temperature-controlled incubator at 20°C for 6 months. Samples were periodically removed and frozen at -20°C until chemical analyses could be performed. Results from this experiment indicated that the colourless polyphenolic compounds in the juice remained stable during storage. The anthocyanin content of the juice samples decreased drastically during storage. A 77 - 88% decrease after 90 days and a 90 - 95% decrease after 180 days in anthocyanin content were measured, respectively. Temperature influenced anthocyanin degradation, as an additional experiment conducted at 4°C, presented a decrease in anthocyanins of only 41 - 58% within 300 days (Will & Dietrich, 2006).

Influence of ascorbic acid on anthocyanin stability

The influence of ascorbic acid on anthocyanin stability has been studied extensively, without irrefutable results (Sadilova *et al*., 2009). García-Viguera and Bridle (1999) found that the

degradation of anthocyanins was independent of ascorbic acid presence during storage. Similar results were found by De Rosso and Mercadante (2007), Marti *et al*. (2001), and Poei-Langston and Wrolstad (1981). Other studies conducted by Rababah *et al*. (2005) showed that ascorbic acid had no conclusive effect on anthocyanins. Jurd (1972) determined that the interaction between ascorbic acid and anthocyanins results in condensation products, such as dehydroascorbic acid, furfurals and H_2O_2, and a decrease in colour intensity. Sarma *et al*. (1997) postulated that metal ion-anthocyanidin-ascorbic acid complexes formed between anthocyanins and ascorbic acid in the presence metal ion. This is believed to protect the anthocyanins from ascorbic acid degradation.

A study conducted by González-Molina *et al*. (2009) on pomegranate juice containing varying levels of lemon juice, indicated a significant decrease in anthocyanin stability with an increase in lemon juice content, independent of the structure of the anthocyanins. Ascorbic acid was noted to have a greater effect on monoglucosides than diglucosides during storage (García-Viguera & Bridle, 1999). The study conducted by González-Molina *et al*. (2009) also indicated flavanone degradation by ascorbic acid. Anthocyanin degradation generally occurred at the beginning of storage. Ascorbic acid could possibly have a bleaching effect on anthocyanins (González-Molina *et al*., 2009).

García-Viguera and Bridle (1999) have reported that oxygen is key to the decolourisation of anthocyanidins by ascorbic acid. The decolourisation of anthocyanidins occurs through the oxidative cleavage of the pyrilium ring of the anthocyanidin by ascorbic acid. Alternatively, the stability of anthocyanin pigments has been found to decrease quicker under oxygen-free conditions than in the presence of oxygen. To explain the loss in colour in the presence of ascorbic acid, the condensation mechanism of anthocyanin degradation is favoured. Changes in the colour of the anthocyanins during storage with ascorbic acid were found to be more dependent on the type of anthocyanin than on the presence of ascorbic acid. The monoglucosides became lighter than the diglucoside anthocyanins (García-Viguera & Bridle, 1999).

SUMMARY

As mentioned, functional foods are defined as “foods or dietary components that may provide a health benefit beyond that of basic nutrition” (Hasler, 1998). These foods are rich in polyphenolic compounds that reportedly have beneficial health properties, such as the ability to relieve or eliminate oxidative stresses that cause damage to macromolecules, such as DNA, proteins and lipids (Ames *et al*., 1993).

Plums are considered to contain large amounts of polyphenolic compounds that can reduce the risk of heart disease and may even improve bone growth (Bridle & Timberlake, 1996). The polyphenolic compounds found in plums include phenolic acids, such as neochlorogenic acid, chlorogenic acid (Donovan *et al*., 1998), flavan-3-ol monomers, procyanidin A and B dimers, and trimers, flavonols, such as quercetin-3-rutinoside, quercetin-3-glucoside, and quercetin-3-xyloside

(Nunes *et al*., 2008), as well as anthocyanins, such as cyanidin-3-glucoside and cyanidin-3-rutinoside, peonidin-3-glucoside, and peonidin-3-rutinoside (Usenik *et al*., 2009). These compounds are found in greater concentrations in the skins of the fruit than in the flesh (Nunes *et al*., 2008). The flavonols, kaempferol-3-rutinoside, kaempferol-3-glucoside, kaempferol-3-galactoside, and kaempferol-3-arabinoside-7-rhamnoside, have been detected in plums in small amounts (Nunes *et al*., 2008).

The stability of antioxidants is influenced by many factors during storage. These factors include storage temperature (Kevers *et al*., 2007), ultraviolet radiation, carbon dioxide, atmospheric oxygen, pH (Kalt, 2005), the type of fruit and cultivar (Piga *et al*., 2003), the presence of certain degrading enzymes or complexing compounds (Will & Dietrich, 2006), as well as the presence of transition metals, such as copper ions, which assist in anthocyanin degradation (García-Viguera & Bridle, 1999).

Descriptive sensory analysis has often been used to compare different products or product formulations (Lawless & Heymann, 1998). Through statistical analyses, the data collected from QDA can be analysed to determine the complete sensory profile of the samples, including the flavour, mouthfeel, aftertaste and visual perspective of the samples. This data can be used to determine whether a product might be accepted by consumers. Consumer sensory analysis is conducted to determine whether product characteristics will be considered acceptable by consumers, whether the consumer likes the product, or whether the consumer prefers the product over other products. Statistical methodology analyses conducted on consumer analysis data include preference mapping, analysis of variance, principal component analysis, factor analysis, and cluster analysis (Lawless & Heymann, 1998).

Plum-based beverages could thus have the potential to compete with high antioxidant fruit beverages, could contribute to the maintenance and improvement of consumer health, and to boot, can be developed to be acceptable to the consumer from a sensorial perspective.

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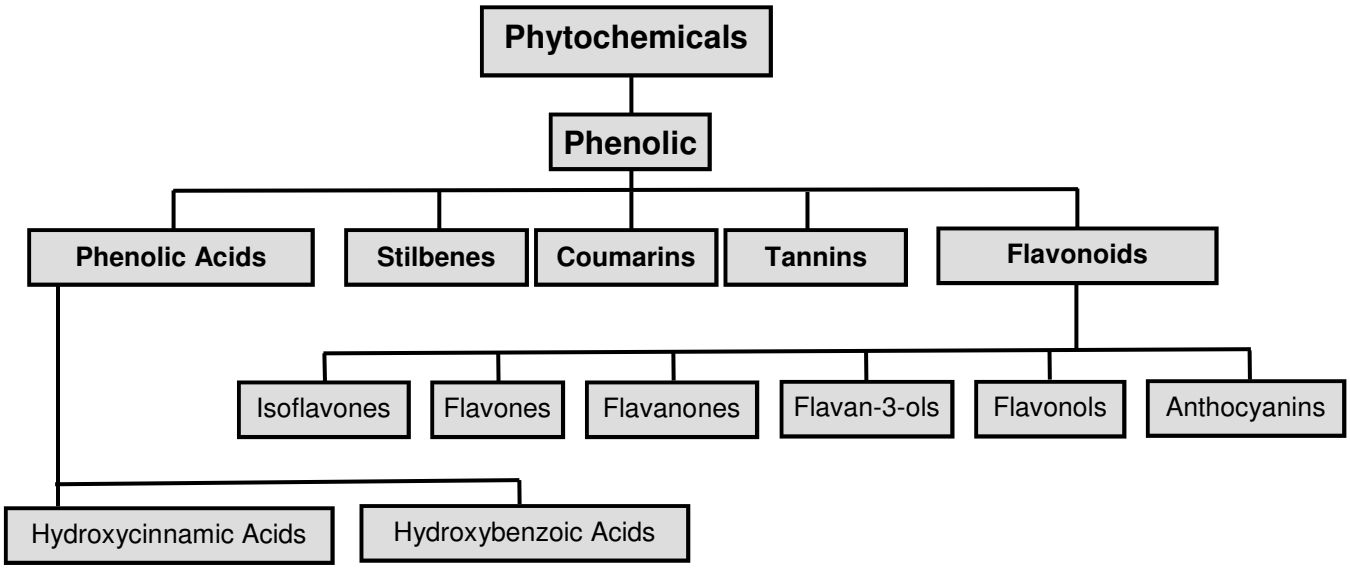
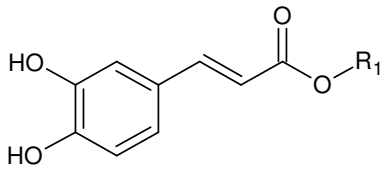


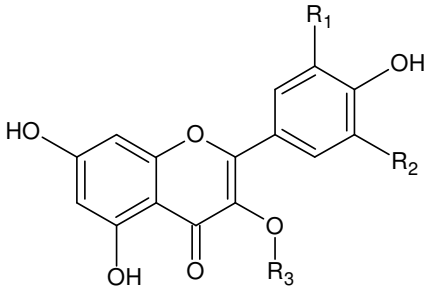
Figure 1. Graphic representation of the various polyphenolic groups found in nature.

Hydroxycinnamic acids

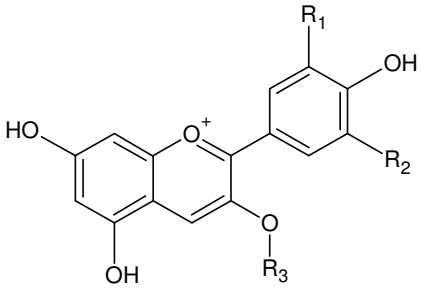


Chlorogenic acid, neochlorogenic acid = R_1 = quinic acid

Flavonols



Anthocyanins

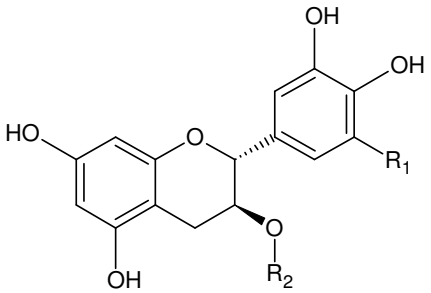


R_1, R_2 = OH, OCH_3 or H, R_3 = mono- or di-saccharide

e.g. quercetin-3-glucoside:
R_1 = OH, R_2 = H, R_3 = glucosyl

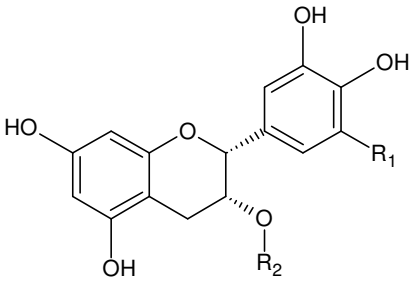
e.g. cyanidin-3-glucoside:
R_1 = OH, R_2 = H, R_3 = glucosyl

Flavan-3-ols



(+)-catechin: R_1 = H, R_2 = H; (+)-gallocatechin: R_1 = OH, R_2 = H

(+)-catechin gallate: R_1 = H, R_2 = gallate; (+)-gallocatechin gallate: R_1 = OH, R_2 = gallate



(-)-epicatechin: R_1 = H, R_2 = H; (-)-epigallocatechin: R_1 = OH, R_2 = H

(-)-epicatechin gallate: R_1 = H, R_2 = gallate; (-)-epigallocatechin gallate: R_1 = OH, R_2 = gallate

Figure 2. The basic chemical structure of the major polyphenolic groups found in plums

Chapter 3

Development and characterisation of a functional beverage from red-fleshed Japanese plums (*Prunus salicina* L.)

ABSTRACT

Twenty-two commercial red fruit beverages, which were considered high in antioxidants, were locally acquired. The total soluble solids content, pH, titratable acidity, colour, antioxidant activity (oxygen radical antioxidant capacity (ORAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP)), total polyphenolic content, total anthocyanin content, and ascorbic acid content of these beverages were determined. Data from the commercial beverages aided product development and was used for benchmarking of five red-fleshed plum nectar formulations containing 0, 8, 16, 24, and 32% red-fleshed plum skin extract. Consumer and descriptive sensory analyses were conducted on the nectar formulations, along with colour, total polyphenolic content, total anthocyanin content, individual polyphenolic compound contents, and antioxidant activity (ORAC, $DPPH^{\bullet}$ scavenging activity, FRAP) analyses. An increase in polyphenolic content and antioxidant activity was observed with an increase in skin extract content of the formulations. Consumer analysis (N=100) indicated that the 0 and 16% skin extract formulations were most preferred by consumers, with no significant ($P>0.05$) difference detected between the 8, 16 and 24% skin extract formulations. The 32% skin extract formulation was least preferred. The sensory analysis descriptors of the formulations were plum and plantlike aroma, plum and plantlike flavour, sweetness, acidity, and astringency. Sensory results indicated an increase in plantlike aroma and flavour, acidity, and astringency, as well as a decrease in plum aroma, plum flavour, and sweet taste with an increase in skin extract content of the nectar formulations. This is postulated to result from an increased total polyphenolic content, which is believed to mask the positive attributes and contribute to the negative sensory attributes of the formulations. The plum nectar formulations possessed higher total polyphenolic content, antioxidant activity, and colour values than the average for the commercial beverages. Red-fleshed plum nectars have the potential to compete with high antioxidant fruit beverages and could contribute to the maintenance and improvement of consumer health.

KEYWORDS Analysis of variance, antioxidants, commercial beverages, plum nectar, sensory analysis, skin extract, polyphenolic compounds, preference, *Prunus salicina* L.

INTRODUCTION

Plums contain a variety of polyphenolic compounds, including hydroxycinnamic acids, such as chlorogenic acid and neocholorgenic acid (Donovan *et al*., 1998), anthocyanins, such as cyanidin-3-gluocside and cyanidin-3-rutinoside (Robards *et al*., 1999), and flavonols, such as quercetin-3-rutinoside, quercetin-3-glucoside and quercetin-3-xyloside (Tomás-Barberán *et al*., 2001). These polyphenolic compounds are antioxidants, which bear many health-promoting properties, such as the prevention or inhibition of cataracts, atherosclerosis (Kehrer & Smith, 1994), and certain cancers (Thomasset *et al*., 2006). Heart disease (Chong *et al*., 2010) and degenerative diseases, such as Parkinson's and Alzheimer's diseases (Aquilano *et al*., 2008), are also believed to be inhibited by these compounds. The benefits of flavonols include their antiulcer, antispasmodic, antisecretory, antidiarrhoeal (Carlo *et al*., 1999) and antihepatotoxic properties (Hemingway & Larks, 1988), as well as their ability to inhibit the human immunodeficiency virus type 1 protease (Agullo *et al*., 1997). Plum juice was found by Shukitt-Hale *et al*. (2009) to inhibit age-related cognitive decline in rats. The health-promoting properties of polyphenolic compounds in fruits have prompted researchers, breeders and manufacturers to embark on research and development of high antioxidant functional food products, especially products processed from high antioxidant fruits.

Although plums and plum beverages are characterised as functional foods rich in polyphenolic compounds and are thus considered to possess many beneficial, health-promoting properties, there are not many commercial plum beverage products currently available on the South African market. The breeding of new red-fleshed plum cultivars, that could contain a higher than average total polyphenolic content, is currently underway at Bienne Donné, a research facility of the Agricultural Research Council (ARC) in South Africa. These cultivars create opportunities for the development of high-antioxidant plum-based functional products.

Currently, limited literature is available on plum juices or nectars regarding sensory properties, polyphenolic content, and antioxidant activity. Information regarding polyphenolic content and antioxidant activity for plums and plum juices vary considerably (Tomás-Barberán *et al*., 2001). This is mainly due to differences between plum species, which complicates the comparison of findings (Tomás-Barberán *et al*., 2001). Kim *et al.* (2003) stated that varying levels of total polyphenolic compounds in plums are due to differences in cultivars, geographic origins, growth conditions, agricultural practices, and differences in analytical methods. Circumstances that affect polyphenolic compounds, and thus the sensory characteristics of plums and plum products, include processing conditions, such as blending or slicing, high pH, high temperature, as well as oxygen and light exposure (Kalt, 2005). These conditions should be taken into account during product development (Kalt, 2005).

There is also a lack of information currently available regarding the sensory characteristics and consumer preference for plum juices. Information on how the levels of polyphenolic

compounds affect consumer preference is also currently limited. This information is important to manufacturers, as an increased level of polyphenolic compounds could lead to the development of the undesirable characteristic, such as astringency and bitterness (Gil *et al*., 2002). Astringency and bitterness are unpleasant mouthfeel and flavour characteristic, respectively, which are often caused by polyphenolic compounds (Brossaud *et al*., 2001). Bitterness and astringency are influenced by the molecular sizes of the polyphenolic compounds. Monomers are considered more bitter than astringent (Brossaud *et al*., 2001). Astringency-causing polyphenolic compounds include flavan-3-ols and hydroxycinnamic acids (Brossaud *et al*., 2001). These compounds are readily found in plums (Gil *et al*., 2002), thus potentially contributing to astringency. Brossaud *et al.* (2001) stated that anthocyanins are known to interact strongly with certain polyphenolic compounds, possibly altering their affinity for salivary proteins. The complex of sensations that occur due to the precipitation of salivary proteins may be altered by interactions between anthocyanins and salivary proteins. The polymerisation and/or condensation of anthocyanins cause a decrease in astringency. Alternatively, astringency is considered to increase with the degree of polymerisation of flavan-3-ol monomers and oligomers, as well as with an increase in the molecular weight of astringency-causing compounds (Brossaud *et al.,* 2001).

During the development of a functional, high polyphenolic compound-containing beverage, regulations set in place by the Department of Agriculture and Fisheries (1980) should be adhered to. Fruit beverages regulated by the Department of Agriculture and Fisheries (1980) include fresh fruit juice, unsweetened juice, sweetened juice, nectar, squash, and drink. 'Fresh juice' is a term reserved for 100% fruit juices with no added preservatives. As per the regulations, a fresh juice should be consumed within 2 h. Unsweetened and sweetened juices are permitted to contain preservatives and natural fruit flavourants, ascorbic acid, and/or carbon dioxide. A sweetened juice may also contain natural and/or synthetic sweeteners. Fruit nectars may contain less than 100% fruit juice. The amount of juice contained by a nectar is determined by the fruit type (typically less than 50%). Fruit drinks typically contain less than 10% fruit juice. For each type of fruit beverage, minimum °Brix values are specified. No specific regulations are mentioned for plum beverages, which are classified as from unspecified fruits. Plum skins, containing a large amount of polyphenolic compounds (Donovan *et al*., 1998) otherwise going to waste, can be processed into a skin extract. The addition of skin extract to plum pulp would allow the formulation of plum nectars with a high polyphenolic content and antioxidant activity.

The aims of the study included the development of a red-fleshed plum juice-based beverage with a high antioxidant capacity and polyphenolic content. Chemical analyses were conducted on the plum nectar formulations and the commercial fruit juices to determine the colour, total polyphenolic content and antioxidant capacity of the formulations and to benchmark the nectar formulations against similar commercially available beverages. A profile of the sensory attributes of the different formulations was conducted, along with consumer preference testing.

MATERIALS AND METHODS

Chemicals

The chemicals used are shown in Table 1.

Commercial fruit beverage samples

Twenty-two commercial fruit beverages were purchased from local supermarkets around Stellenbosch. These beverages all contained juice from red, violet, and blue fruits, such as blueberries, cranberries and pomegranates, which are all considered to have a high polyphenolic content. The label information of these beverages is shown in Table 2. These beverages were characterised in terms of polyphenolic composition, antioxidant activity, and colour, as well as, titratable acidity, total soluble solids content and pH analyses. These characteristics served as guidelines for the development of the functional red-fleshed plum nectars.

Product development

A test batch of fruit (35.6 kg) consisting of different yellow- and red-fleshed Japanese plum cultivars (*Prunus salicina* L.) was obtained from Bienne Donné farm (Groot Drakenstein, South Africa; S 33.84, E 18.98) in January 2009 and stored at 10°C until deemed eating ripe. The plums were initially washed and weighed before being cut in half and the pits removed, to facilitate pulping. The halved plums were pulped using a pilot scale hammer mill before being sent through a juice-skin separator. The separator separates the pulp from the skins by means of a sieve. These processes were conducted under carbon dioxide to inhibit oxidative browning and the loss of antioxidant activity. Thereafter, the plum pulp was pasteurised at approximately 72°C for 15 s. The skins (3.0 kg) and pulp (18.7 kg) retrieved from the milling and separating processes were packaged in 200 mL foil sachets before being cooled in cold water, labelled and stored in a freezer at -15°C.

An identical milling and separating process was implemented on the newly developed red-fleshed plum selection, PR05-17, obtained from Bienne Donné in January 2009. The fruits (24.7 kg) were harvested (15 January 2009) at commercial ripeness (6-8 kg firmness) and were cold stored using a commercial dual temperature cold storage regime (10 days at -0.5°C, 9 days at 7.5°C; 10 days at -0.5°C), followed by seven days of ripening at 10°C before pulping, as described above. The firmness, total soluble solids content, and acidity of the ripe fruits were 3.7 kg, 14.4 °Brix, and 26.8 g malic acid/L, respectively. After pulping 14.5 kg pulp and 3.4 kg skins were obtained.

Multiple beverage formulations were developed from the test batch plum pulp and skins as there were only limited amounts of the PR05-17 selection available. The calculations used to determine the amounts of formulation ingredients are shown in Table 3 (Personal communication, Dr C. F. Hansmann, ARC Infruitec-Nietvoorbij, Stellenbosch). The optimum formulation was

selected by an in-house expert panel. Formulations (13 °Brix) with 30, 35 and 40% pulp, varying amounts of apple concentrate (sourced from Pacmar, Worcester, South Africa), and 0% skin extract were firstly developed. Thereafter, the optimum total soluble solids content was determined by preparing formulations with 40% pulp. Government regulations state that a beverage formulation from an unspecified fruit may be called a nectar rather than a juice blend when it contains 40% pulp (Department of Agriculture and Fisheries, 1980). The total soluble solids contents that were tested were 12, 12.5, 13 and 14 °Brix. Government regulations for an unspecified fruit nectar stipulate that the nectar formulation should have a minimum sugar content of 12 °Brix or more (Department of Agriculture and Fisheries, 1980). The total soluble solids content of the pulp and formulations were verified using a refractometer (Atago Palette PR-101; Tokyo, Japan). Different juice concentrates were tested using 40% pulp. The pH, total soluble solids content, titratable acidity, and the providers of the concentrates are shown in Table 4. The juice concentrates were evaluated by an in-house expert panel based on their effects on the flavour, colour, acidity, astringency, and sweetness of the nectar formulation.

A method for the preparation of a plum skin extract was developed. Five methods for skin extraction were attempted. Firstly, the skin and deionised water (1:2) was stirred for 5 min and centrifuged for 10 min at 8000 rpm before undergoing vacuum-pump filtration. The remaining skins were then added to more deionised water (1:1) and the process was repeated. The second attempt included the blending of the skins with deionised water (1:2) for 1 min before subjecting the blend to vacuum-pump filtration. The third attempt included the blending of the skins with deionised water (1:2) for 1 min and centrifugation at 8000 rpm for 10 min before vacuum-pump filtration. The fourth attempt was similar to the third, but nitrogen gas was introduced to the mixture of deionised water and skins during blending. This was done to determine whether the introduction of oxygen by blending degraded the anthocyanins in the skin extract considerably. The fifth attempt was, again, similar to the fourth attempt except that the skin to deionised water ratio was decreased to 1:1.

Nectar formulations

The final formulations that were developed contained red-fleshed plum pulp (40%), grape concentrate from Koöperatieve Wijnbouwers Vereniging (KWV, Paarl, South Africa), deionised water and varying amounts of skin extract (0, 8, 16, 24, and 32%). These formulations were prepared approximately 2 h before sensory and consumer analyses were conducted. The final total soluble solids content of the nectar formulations was 13 °Brix.

Chemical and physical analyses

Samples were centrifuged at 14000 rpm in a Boeco M-24A centrifuge (Hamburg, Germany) to remove solids before all analyses. All spectrophotometric measurements were performed using a BioTek Synergy HT microplate reader with Gen5 software for data acquisition (Vermont, USA).

Colour analysis

Absorbance measurements of the nectar formulations at 520 nm were used as an indication of red colour, while the sum of absorbance measurements from 370 to 700 nm measured every 10 nm for each formulation were used as an indication of total colour. Absorbance measurements of 200 µL of each undiluted sample were measured in triplicate in a clear polystyrene 96-well microplate.

Firmness analysis

The firmness of the plums was measured using a standard manual penetrometer with a probe diameter of 11 mm. The force used to penetrate the fruit to a standard depth was measured in kg.

Total soluble solids analysis

The total soluble solids content of the pulp, commercial beverage samples, skin extract, and nectar formulations was measured in °Brix using an Atago Palette PR-101 refractometer (Tokyo, Japan).

pH analysis

The pH of the pulp, commercial beverage samples, skin extract, and nectar formulations was determined using a Crison GLP 21 pH meter (Barcelona, Spain). The pH meter was calibrated with the pH 4 and pH 7 Sigma buffer reference standards (pH 7 ± 0.01 at 25°C and pH 4 ± 0.01 at 25°C).

Titratable acidity analysis

The titratable acidity of the juice concentrates, commercial beverage samples, pulp, skin extract, and nectar formulations was determined using an automatic titrator (Crison Compact Titrator, Version D; Alella, Spain). The titratable acidity of the samples was measured in g malic acid/L.

Ferric reducing/antioxidant power (FRAP) assay

The reagent required for the ferric reducing/antioxidant power assay analysis (modified from Benzie & Strain, 1996) is prepared by mixing 100 mL freshly prepared 300 mM acetate buffer (pH 3.6), 10 mL of 10 mM TPTZ (2,4,6- tris(2-pyridyl-*s*-triazine)) in 40 mL HCl, and 10 mL of 20 mM $FeCl_3$. The standard stock solutions were prepared using 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) with a concentration range of 50 - 600 µmol/L. The nectar formulation and commercial beverage samples were adequately diluted using deionised water. Triplicate aliquots (20 µL) of the samples, standards, and blanks (dH_2O) were pipetted into allocated wells in a 96-well microplate. The FRAP solution (180 µL) was then added to the wells using a multichannel pipette. Spectrophotometric measurements were taken at 593 nm to determine the formation of a coloured TPTZ-Fe^{2+} complex. These increases in absorption were measured after 4 min at 37°C. The results are expressed as µmol Trolox equivalents (TE)/L sample.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH$^{\bullet}$ scavenging assay used was a modified version of the method by Rangkadilok *et al.* (2007). The DPPH$^{\bullet}$ solution was prepared by dissolving 3.94 mg of the DPPH in 100 mL methanol. This solution were freshly prepared, sonicated, covered in aluminium foil, and kept in the dark until use. Trolox standard stock solutions were prepared with a concentration range of 50 – 400 µmol/L. The nectar formulation and commercial beverage samples were appropriately diluted using deionised water. The sample standards and blanks (25 µL) were pipetted in triplicate into the allocated wells of the deep-well microplate. Subsequently, the DPPH solution (255 µL) was added to all the wells of the deep-well plate and a silicon mat was used to seal the plate, preventing methanol evaporation. Thereafter, the plate was incubated in a dark cupboard for 2 h. After incubation, 200 µL of the reaction mixture was pipetted into a 96-well flat-bottom microplate and vortexed with a MixMate (30 s, 1000 rpm) (Merck; Germiston, South Africa). An absorbance measurement was taken at 515 nm. A similar plate was prepared with methanol replacing the DPPH$^{\bullet}$ solution to serve as a sample blank. The absorbance results from the methanol plates were subtracted from the DPPH plate results to remove the influence the pigments in the samples had on the absorbance readings. The results for the DPPH$^{\bullet}$ scavenging activity assay were expressed as µmol Trolox/L sample.

Oxygen radical antioxidant capacity (ORAC) assay

The ORAC method used was a modification of the method of Huang *et al.* (2002). This assay determines the ability of antioxidants in the samples to protect a specific fluorescence indicator, which is introduced during the assay, from oxidative degradation (Zulueta *et al*., 2009). The degradation of the indicator is detected through a decrease in fluorescence (Zulueta *et al*., 2009). A thermal barrier was created by adding 300 µL of deionised water to the peripheral wells of a black fluorescence microplate with a clear bottom (Huang *et al*., 2002). The appropriately diluted samples, standards and blanks (25 µL) were pipetted in triplicate into the allocated wells of the plate before the fluorescence working solution (150 µL), which was prepared by diluting the fluorescein disodium stock solution (3 mg/100 mL in 75 mM potassium phosphate), was added to these wells. The standards used for this assay were prepared from a stock solution of Trolox (5 – 30 µmol/L) and the blanks were deionised water. A 2, 2'-azo-bis-(2-amidinopropane) dihydrochloride (AAPH) solution was prepared by dissolving 0.414 g AAPH to 10 mL with the potassium phosphate buffer. The AAPH was automatically dispensed in the wells by the microplate reader. Before the AAPH was added, the plate was incubated for 10 min. Fluorescence readings of each well were taken every 1 min for 35 min after the addition of AAPH. The fluorescence readings had an excitation wavelength of 485 nm and an emission wavelength of 530 nm. The AAPH was kept on crushed ice to avoid thermal degradation and the fluorescence working solution was kept in a dark cupboard to avoid degradation from ultraviolet light. Results are expressed as µmol TE/L. The ORAC value is calculated by firstly calculating the net area under the curve of the reference

standards and the samples. The net area under the curve is calculated by subtracting the area under the curve of the blank from that of the sample. The area under the curve for Trolox concentration is calculated in a similar fashion. The standard curve is, thereafter, calculated by plotting the Trolox concentrations against the average of the net area under the curve of two measurements for each Trolox concentration. The ORAC values are subsequently calculated through the regression equation between the Trolox concentration and the net area under the curve (Huang *et al*., 2002).

Total polyphenolic content analysis

The total polyphenolic content of the nectar formulations and the commercial beverage samples was determined using a modified version of a method by Singleton and Rossi (1965). Initially, the samples were appropriately diluted with deionised water. A gallic acid stock solution was prepared and diluted with deionised water to give a standard series of 10, 20, 40, 60 and 100 mg/L. A 10% (v/v) aqueous solution of the Folin-Ciocalteau reagent and a 7.5% (w/v) aqueous solution of sodium carbonate were used. Aliquots (20 µL) of the sample dilutions, the standards and the deionised water controls were pipetted in triplicate into assigned wells of a 96-well microplate. Thereafter, a Gilson multichannel pipette was used to add 100 µL of the diluted Folin-Ciocalteau reagent and 80 µL of the sodium carbonate solution to the wells, respectively. The microplate was then vortexed with a MixMate (30 s, 1000 rpm) (Merck; Germiston, South Africa) and incubated for 2 h at 32°C before the absorbance values were read (765 nm). The standard series allows for the total polyphenolic content of the samples to be calculated. The results are expressed in mg gallic acid equivalents (GAE)/L sample.

Total anthocyanin content analysis

The method of Ribéreau-Gayon and Stonestreet (1966) was followed with minor modifications. The samples (50 µL) were pipetted into the wells of a microplate in triplicate. A multichannel pipette was used to add the 2% HCl solution (150 µL). Thereafter, the plate was vortexed using a MixMate (30 s; 1000 rpm) (Merck; Germiston, South Africa) and incubated at ambient temperature for 10 min. The absorbance was measured at 515 nm and 700 nm to correct for possible turbidity. Total anthocyanin content was calculated as mg cyanidin-3-glc equivalents/L by using its extinction coefficient (ε = 25965 $M^{-1}.cm^{-1}$; Cevallos-Casals *et al*., 2006).

HPLC analysis of total polyphenolic content

The HPLC analysis of the major polyphenolic compounds in plum nectars was performed using an Agilent 1200 series HPLC (Waldbronn, Germany), including a quaternary pump, autosampler, column thermostat, diode-array detector. Chemstation software for LC 3D systems Rev. B.02.01 [244] (Agilent) was used for data acquisition and analysis. A Gemini-NX C18 (3 µm particle size, 110 Å pore size, 150 x 4.6 mm, Phenomenex (Santa Clara, CA, USA)) column, protected by a

guard column with the same stationary phase, was used. The column was kept at a constant temperature of 30°C. Samples were injected in duplicate using an injection volume of 50 µL. Mobile phases were 7.5% formic acid in water (A) and 7.5% formic acid in acetonitrile (B) with a flow rate of 1 mL/ min. The mobile phase gradient is shown in Table 5.

The major peaks were identified as cyanidin-3-glucoside (quantification wavelength 520 nm), cyanidin-3-rutinoside (quantification wavelength 520 nm), quercetin-3-glucoside (quantification wavelength 350 nm), quercetin-3-rutinoside (quantification wavelength 350 nm), quercetin-3-xyloside (quantification wavelength 350 nm), and chlorogenic acid (quantification wavelength 320 nm) by comparing retention times and UV-Vis spectra with that of authentic reference standards (Table 1). Calibration curves for cyanidin-3-glucoside (0.07 – 1.50 µg injected), cyanidin-3-rutinoside (0.05 – 1.00 µg injected), quercetin-3-glucoside (0.04 – 0.35 µg injected), quercetin-3-rutinoside (0.02 – 0.35 µg injected) and neochlorogenic acid (0.03 – 0.60 µg injected) run before every analysis were used for external calibration. Quercetin-3-xyloside was tentatively identified using liquid chromatography with tandem mass spectrometry (Central Analytical Facility, Stellenbosch University, Stellenbosch, South Africa) and quantified as quercetin-3-glucoside equivalents. Neochlorogenic acid was quantified using the calibration curve for chlorogenic acid and a conversion factor. The conversion factor was calculated by comparing the slopes for the calibration curves for chlorogenic acid and neochlorogenic acid.

Contribution of ascorbic acid to antioxidant activity

The HPLC analysis of the ascorbic acid content in plum nectar formulations and commercial beverage samples was performed using the same equipment and column as the HPLC analysis of the polyphenolic compounds. Samples were injected in duplicate using varying injection volumes, depending on the ascorbic acid content. Mobile phases were 0.1% formic acid in water (A) and acetonitrile (B), with a flow rate of 0.8 mL/min. The mobile phase gradient is shown in Table 5. The ascorbic acid peak (quantification wavelength 254 nm) was identified by comparing the retention time and UV-Vis spectra of the samples with that of an authentic reference standard (Table 1). A calibration curve for ascorbic acid (0.1 – 0.9 µg injected), which was run before the analysis, was used for external calibration.

To determine the contribution of ascorbic acid on the $DPPH^{\bullet}$ scavenging activity, total polyphenolic content, FRAP, and ORAC analyses, different concentrations of ascorbic acid and the respective reference standards were prepared and analysed using these methods. A response method factor was determined for each assay to calculate the contribution of ascorbic acid determined from the results. A response factor for ascorbic acid in each assay was calculated by dividing the slope of the dose-response curve for ascorbic acid by the response curve of the respective reference standard. The response factor of each assay was multiplied with the respective ascorbic acid concentrations. These values were subtracted from the original $DPPH^{\bullet}$

scavenging activity, total polyphenolic content, FRAP, and ORAC analyses results to indicate the values without the contribution of ascorbic acid in these analyses.

Sensory analysis

Descriptive sensory analysis was performed using an eight-member trained panel of judges to determine the sensory attributes of the formulations (Lawless & Heymann, 1998). The training of the panel of judges was achieved with the consensus method in order to enhance the consistency of the trained panel (Lawless & Heymann, 1998).

A 100 mm unstructured line scale was used for analysing the sensory descriptors of the five plum nectar formulations. These attributes were rated from *None* to *Prominent* (0 - 100). The descriptors included plum and plantlike aroma, plum and plantlike flavour, sweetness, acidity, and astringency. Two training sessions of approximately 4 h in length were used where the nectar formulations were analysed among the panel to reach a consensus on the level of the attributes of the respective scales. The references used during the training of the panel are shown in Table 6.

The nectar samples were served at room temperature in standard ISO wine tasting glasses in a temperature-controlled room (21°C). Approximately 40 mL of each sample was decanted into the wine glasses and covered with a plastic lid. The judges received water and unsalted fat-free water biscuits to cleanse their palates between samples. The samples were presented in a randomised order with three-digit random codes on each glass. An example of a the paper ballot is shown in Addendum 1.

Consumer liking

The nine-point hedonic scale was used in the assessment of the consumers' degree of liking of the five nectar formulations. This method has been considered easy to use and widely acceptable (Lawless & Heymann, 1998). The scale has been found to offer extremely reliable results, but is, unfortunately, dependent on the size of the panel. The consumers were asked to rate their degree of liking of the various samples on the scale. The nine-point hedonic scale consists of the following nine categories: *9 = Like extremely, 8 = Like very much, 7 = Like moderately, 6 = Like slightly, 5 = Neither like nor dislike, 4 = Dislike slightly, 3 = Dislike moderately, 2 = Dislike very much, 1 = Dislike extremely* (Lawless & Heymann, 1998).

Approximately 20 mL of nectar was served at ambient temperature (ca 21°C) in Styrofoam™ cups. The plum nectar formulations were freshly prepared approximately 2 h before the consumer panel testing. Random three-digit codes were used to codify the formulations. The formulations were served in a complete randomised order. Water was provided for the cleansing of the palate. The room in which the tasting was conducted was kept at a constant temperature (21°C).

The consumers were not asked to give a reason for their preference (Lawless & Heymann, 1998). The hedonic scale allowed for both preference and acceptance testing. Consumers (N=100)

of 18 years and older were sourced for the consumer analysis of the plum nectar formulations. These consumers were asked to indicate their gender, age group, and the frequency with which they consume fruit-based beverages. The age groups used in this questionnaire (Addendum 2) were 18 to 25, 26 to 30, 31 to 45, and 46 and older. The frequency of beverage consumption categories were seven times a week or more, three times a week, once a week, twice a month, less than once a month, and never.

Statistical analysis of data

A randomised complete block design was used with five treatments and six replications for the sensory and chemical analyses. The consumer, sensory and chemical data were subjected to test-retest analyses of variance (ANOVA) using SAS® software (Version 9; SAS® Institute Inc, Cary, USA). ANOVA was conducted on the sensory data to test for reliability (Judge*Replication interaction) and internal consistency (Judge*Level interaction). Single, nonconforming judges were identified and removed using the SAS® line plots. These plots indicate temporal stability and internal consistency. The Shapiro-Wilk test was used to test the level of non-normality in the consumer, sensory and chemical data (Shapiro & Wilk, 1965). If non-normality was significant ($P \leq 0.05$), it caused skewness. The outliers were identified and removed until the data were normally distributed (Glass *et al*., 1972). Student's t-tests for the least significant difference (LSD) were calculated for the consumer, sensory and chemical data at the 5% significance level. These least significant differences were used to compare treatment means.

Multivariate statistical analysis was performed using the XLSTAT software (Version 7.5.2; Addinsoft, New York, USA). Discriminant analysis (DA) was conducted on the sensory and chemical data. Principal component analysis (PCA) was conducted on the chemical data to determine possible associations between attributes and samples.

RESULTS AND DISCUSSION

Commercial beverage samples

Commercial beverages containing juice from red, violet, and blue fruits that are considered to be high in polyphenolic compounds were obtained from various stores throughout Stellenbosch. The beverages were mostly berry-based, with cranberry and aronia berry being most prominent in these beverages (Table 2). No commercially available plum beverages were found. The commercial beverages were analysed for total soluble solids, pH, and titratable acidity (Table 7). The commercial beverages had a total soluble solids content between 8.3 and 15.3 °Brix, while the pulpy beverage blends (CS 4, 10, 11, 12, 13, 17, 20, and 22) ranged from 12.7 to 15.3 °Brix. The pH values for the commercial beverages were between 2.7 and 3.6, while the titratable acidity of these beverages was between 3.0 and 18.4 g malic acid/L.

The twenty-two commercial beverage samples made from high polyphenolic compound-containing fruits were used to benchmark the plum nectar formulations (Table 7). The samples were analysed to determine the ranges of antioxidant activity and polyphenolic content of the commercially available beverages for comparison with the plum nectar formulations. Many of the samples processed from high polyphenolic-containing fruits showed a high antioxidant activity and polyphenolic content as expected, while others claimed to be prepared from high polyphenolic compound-containing fruits, but showed a low polyphenolic content and antioxidant activity. These beverages were either not 100% pure juice or only contained a small proportion of the high polyphenolic-containing fruit with the rest of the beverage consisted of low polyphenolic-containing fruit-based juices. Other samples contained ascorbic acid as a preservative. The ascorbic acid contributes to increased FRAP, $DPPH^{\bullet}$ scavenging activity, ORAC, and total polyphenolic values. Ascorbic acid analysis was conducted and corrections for the contribution of ascorbic acid were made to values for beverages containing ascorbic acid (CS 2, 4, 5, 7, 8, 10, 12, 17, 19, and 22) to determine the right antioxidant activity and total polyphenolic content of the commercial beverages.

Product development

The initial product development was performed using a test batch plum pulp. The formulations were analysed by an in-house expert panel. The test batch pulp was used because only limited amounts of fruit from the PR05-17 selection was available. Pulp and skin extract from the PR05-17 selection was used to prepare the formulations after completion of product development. The physicochemical characteristics of the plum pulp and the PR05-17 skin extract are shown in Table 8. The in-house panel found the 40% pulp formulation to be optimum. The formulations with lower amounts of pulp were watery and lacked plum flavour.

The optimum total soluble solids content of the formulation was chosen as 13 °Brix, as this formulation was deemed to possess the perfect balance of acidity and sweetness for this type of nectar by an in-house expert panel. This complies with South African government regulations for nectars from unspecified fruits, which should contain a minimum total soluble solids content of 12 °Brix (Department of Agriculture and Fisheries, 1980). The final total soluble solids content chosen for the plum nectar formulations was similar to the average total soluble solids content of the commercial beverages.

Different juice concentrates (Table 4) were tested using 40% pulp at 13 °Brix. The grape concentrate, provided by KWV, Paarl, was preferred due to its low acidity and its low impact on the flavour of the nectar compared to the other concentrates.

The accumulation of polyphenolic compounds in most fruits is greater in the skin than the flesh (Macheix *et al.*, 1990). To increase the total polyphenolic content in the plum nectar formulations, skin extract was added to the nectar formulation as a substitute for water. Various methods were employed in this study to develop an optimum skin extraction method. The introduction of nitrogen in the development of the skin extract was found to have no effect on the

degradation of the anthocyanins. This result was not surprising, as nitrogen is less dense than air, thus the nitrogen failed to push all of the air out of the blender during processing. Oxygen thus still interacted with the anthocyanins during blending, decreasing anthocyanins (Piga *et al*., 2003). The lack of effect of the nitrogen and the effort and cost involved in using nitrogen gas resulted in this extraction method not being used. The optimum method for the extraction of polyphenolic compounds from the plum skins was the extraction where skins and water (1:2) were blended for 1 min, centrifuged at 8000 rpm for 10 min, and filtered using a vacuum-pump. This method yielded a skin extraction with the highest polyphenolic content compared to the other methods that were developed (data not shown).

The relative amounts of pulp and skin obtained from PR05-17 plum fruits gives a broad indication that the maximum feasible percentage of skin extract in a formulation is 24%. Therefore, the formulations chosen for further testing were the 0, 8, 16, 24 and 32% skin extract formulations. The 32% skin extract formulation was prepared as an extreme case to determine the effects that extreme levels of skin extract could have on the sensory attributes of the plum nectar. The titratable acidity of the nectar formulations ranged from 10.1 to 10.8 mg/L and the pH ranged from 3.33 to 3.30 (Table 9). These results were similar to results of the commercial beverages, which had an average titratable acidity of 8.7 g malic acid/L and pH of 3.27 (Table 7).

Nectar formulations

The 0, 8, 16, 24, and 32% skin extract plum nectar formulations were analysed for colour, antioxidant activity, and polyphenolic composition. The total and red colour of red-fleshed plum nectars showed similar trends, with values increasing with an increase in skin extract content (Table 9). In terms of total and red colour, no significant ($P>0.05$) differences were observed between formulations containing 0, 8, and 16% skin extract. The total and red colour of the 24 and 32% skin extract formulations were similar, but significantly ($P\leq0.05$) higher than that of the 0% skin extract formulation. These results were expected, as an increase in anthocyanin content from an increase in skin extract content, enhances the red pigment of the formulations (Will & Dietrich, 2006). The increase in anthocyanin content from an increase in skin extract content results from the leaching of anthocyanins from the plum skins during skin extract preparation (Will & Dietrich, 2006).

In terms of antioxidant activity, similar results were obtained for the ORAC, $DPPH^{\bullet}$ scavenging activity, and FRAP assays (Table 9). In all cases, the lowest and highest activities were observed for the nectars with the lowest and highest skin extract contents, respectively. In all antioxidant assays, there were no significant ($P>0.05$) differences between 0, 8, and 16% skin extract formulations. Similarly, the values of the ORAC and FRAP assays for the 24 and 32% skin extract formulations were not significantly ($P>0.05$) different. The lack of significant difference between these formulations for FRAP assay was due to the small differences in the skin extract content of the formulations and the insensitivity of the assay. The $DPPH^{\bullet}$ scavenging activity

results indicate that the skin extract contents of the formulations make a limited contribution to the DPPH• scavenging activity of the formulations, as only the 32% skin extract formulation showed a significantly ($P \leq 0.05$) higher activity than the 0% skin extract formulation.

The total polyphenolic and total anthocyanin contents of plum nectar formulations showed an increasing trend with an increasing skin extract content (Table 9), although significant ($P > 0.05$) differences were not observed for each individual formulation.

The content of individual anthocyanins and the individual flavonols (Table 9) increased for increased skin extract content, with each formulation differing significantly ($P \leq 0.05$) from every other formulation. The increase in neochlorogenic acid content from the 0% skin extract formulation to the 32% skin extract formulation was less prominent, but still with significant differences between the formulations. HPLC is a highly sensitive, quantitative technique, explaining these results. Figure 1 shows an example of HPLC chromatograms of a 0% skin extract formulation at different wavelengths.

Comparison of plum nectars with commercial beverages

The total colour measured for the commercial beverage samples (Table 7) ranged from 4.6 to 19.7 absorbance units (AU), with the average total colour being 9.0 AU. The individual measurements were within the linear ranges for the spectrophotometer. This extensive range was due to the large variation in the types of fruit beverages used, ranging from grapefruit beverages (CS 3 and 22), which were light in colour, to a blueberry beverage (CS 1), which possessed a opaque, dark blue colour. The total colour measured for the nectar formulations (Table 9) ranged from 12.7 to 15.6 AU, which was higher than the average determined for the commercial beverages. The variation in the colour of the nectar formulations was exclusively due to the amount of skin extract in each formulation. The red colour was between 0.1 and 0.9 AU for the commercial beverages. This range is large, as some of the commercial beverages tested were prepared from fruits that are rich in red pigments, such as cranberries and pomegranates (CS 2, 5, 6, 7, 8, 9, 11, 14, 15, 17, and 18). The average red colour for these samples was 0.3 AU. The red colour for the plum beverage formulations was between 0.5 and 0.7 AU. These results are higher than the average for the commercial beverages.

The antioxidant activities of the commercial beverages were measured using the FRAP, DPPH• scavenging activity, ORAC, and total polyphenolic content analyses (Table 7). The results for the DPPH• scavenging activity, FRAP, ORAC, and total polyphenolic content assays were influenced by ascorbic acid. A response factor for ascorbic acid in each assay was calculated and the contribution of ascorbic acid was subtracted. Ascorbic acid was detected in ten of the twenty-two commercial beverages, while no ascorbic acid was detected in the plum nectar formulations. The contribution of ascorbic acid to the DPPH• scavenging activity, FRAP, ORAC, and total polyphenolic content analyses results (Table 7) were extensive in some of these beverages, with the contribution of ascorbic acid ranging from 5.4% to 73.2 % for FRAP, and from 0.7% to 40.1%

for DPPH• scavenging activity. The contribution of ascorbic acid ranged from 0.02% to 6.2% for ORAC, and from 0.08% to 8.8% for the total polyphenolic content of the plum nectar formulations. Only values after correction for ascorbic acid content will be discussed further.

The FRAP analysis of the commercial beverages yielded a range of 8 to 560 µmol TE/L. The average value determined for the FRAP analysis of the commercial beverages was 176 µmol TE/L. The range for the FRAP analysis of the nectar formulations (0, 8, 16, 24 and 32% skin extract formulations) was 319 to 373 µmol TE/L, which was higher than the average value determined for the commercial beverages. FRAP values determined by Wanjiku (2009) fell between 2521 to 4715 µmol TE/L for commercial fruit juices, such as raspberry, cranberry and loganberry.

The commercial beverages results (Table 7) for DPPH• scavenging activities ranged from 211 to 5518 µmol TE/L. The plum nectar results are higher than the average (2085 µmol TE/L) of the commercial beverage results for DPPH• scavenging activity. The range for DPPH• scavenging activity of red fruit beverages by Wanjiku (2009) was determined as 2076 to 4146 µmol TE/L. The DPPH• scavenging activity of the formulations thus fell within the range of Wanjiku (2009) for red fruit beverages. The results from the study by Wanjiku (2009) and the formulations results were higher than the DPPH• scavenging activity for the commercial beverages (Table 7) analysed in the current study.

The average for the ORAC results (Table 7) of the commercial beverages was 10798 µmol TE/L. The ORAC results of the commercial beverages ranged from 2238 to 22929 µmol TE/L. The plum nectar formulations ranged from 18597 to 20735 µmol TE/L (Table 9), which was higher than the average result for the commercial beverages (Table 7). Wanjiku (2009) determined a range of 7620 to 18874 µmol TE/L for the ORAC assay conducted on red fruit beverage samples. The commercial beverages from this study thus fell within the range determined by Wanjiku (2009), while the nectar formulations were above this range.

The total polyphenolic content of the commercial beverages (Table 7) ranged from 54 to 862 mg GAE/L. The total polyphenolic content of the nectar formulations (Table 9) ranged from 437 to 536 mg GAE/L, which was higher than the average (432 mg GAE/L) for the commercial beverages. This indicates that the plum nectar formulations were on par with commercially available high polyphenolic-containing fruit beverages with regards to the total polyphenolic content. Wanjiku (2009) determined that commercial red fruit beverages ranged from 191 to 611 mg/L total polyphenolic content. These results fell within the range of the commercial beverages analysed in this study (Table 7). The five plum nectar formulations (Table 9) fell within the upper part of the range for total polyphenolic content of the commercial beverages examined by Wanjiku (2009). According to Chang *et al.* (1994), the total polyphenolic content of plum juices ranged from 270 to 4170 mg GAE/L. These juices were, however, prepared by adding 0.2% Clarex® L (Solvay Enzymes Inc., Elkhart, USA) before blending at 49°C for 3 h (Chang *et al.,* 1994). Bentonite and gelatine were added to the juice for juice clarification and the mixture was left overnight.

Thereafter, the mixture underwent racking, filtration, and pasteurisation (Chang *et al.,* 1994). The five plum nectar formulations prepared in this study fell within the range of plum juices analysed by Chang *et al.* (1994). Shukitt-Hale *et al*. (2004) measured a plum juice as having a total polyphenolic content of 1264 mg GAE/L, which was much higher than that of the developed nectar formulations of the current study. Juice prepared in the study by Shukitt-Hale *et al.* (2009) was 100% pure plum juice, while the nectar formulation of the current study only contained 40% pulp, explaining the higher results obtained by Shukitt-Hale *et al.* (2009).

The total anthocyanin content of the commercial beverages (Table 7) was determined as 4 to 44 mg cy-3-glc/L. The average total anthocyanin content of the commercial beverage samples was 12 mg cy-3-glc/L. The values for this measurement, determined for the plum nectar formulations, were between 14 and 20 mg cy-3-glc/L. The total anthocyanin content of the plum nectar formulations (Table 9) was higher than the average of the total anthocyanin content of the commercial beverages. The total anthocyanin content of the nectar formulations prepared in this study was higher than the red fruit commercial juices that were analysed by Wanjiku (2009) and Shukitt-Hale *et al.* (2009).

In summary, red-fleshed plum nectar formulations without skin extract (Table 9) showed similar values for total polyphenolic content and $DPPH^{\bullet}$ scavenging activity compared to average commercial beverages, while higher than average values were observed for the total colour, red colour, the ORAC assay, the FRAP assay, and total anthocyanin content (Table 7 and 9). All values for formulations with skin extracts were higher than the average for commercial beverages. Especially high values were obtained for red-fleshed plum nectar formulations in the ORAC assay, with the 32% skin extract formulation giving a higher value than the maximum for the commercial beverages.

Sensory attributes

The sensory attributes analysed were plum and plantlike aroma, plum and plantlike flavour, sweetness, astringency, and acidity. The sensory results for the five nectar formulations (Figure 2) show significant ($P \leq 0.05$) differences between the various formulations for the respective flavour and mouthfeel attributes. This was expected, as the formulations varied in skin extract content. The skin extract was high in polyphenolic compounds, which could have contributed to the level of astringency (Singleton & Nobel, 1976).

With respect to the plum aroma of the nectar formulations (Figure 2), a decreasing trend was detected with an increase in skin extract content of the formulations. No significant ($P > 0.05$) difference between the 0 and 8% skin extract formulations was found. A significant ($P \leq 0.05$) difference was detected between the 0 and 8% skin extract formulations, and the remaining three formulations (16, 24 and 32% skin extract formulations) for plum aroma. Similar results were obtained for plum flavour and sweetness.

The plantlike aroma, plantlike flavour, acidity, and astringency of the nectar formulations (Figure 2) had an increasing trend with an increase in skin extract content of the formulations. No significant ($P>0.05$) difference was determined between the 0 and 8% skin extract formulations for plantlike aroma. A significant ($P\leq0.05$) difference was found between these formulations and the remaining three formulations, and also between the 16, 24, and 32% skin extract formulations for plantlike aroma. Similar results were found for plantlike flavour, acidity, and astringency. An increase in astringency was expected, as the polyphenolic content of the formulations increased with an increase in skin extract content. Increases in total polyphenolic content are considered to contribute to astringency (Singleton & Nobel, 1976). A study conducted by Brossaud *et al.* (2001) showed that flavan-3-ols and hydroxycinnamic acids, which are readily found in plums, also possibly contribute to astringency (Gil *et al.*, 2002), i.e. dry, puckering mouthfeel (Brossaud *et al.,* 2001).

The PCA biplot (Figure 3) showed a definite separation between the positive sensory attributes, plum flavour, sweetness and plum aroma and the negative attributes, plantlike flavour, plantlike aroma, acidity, and astringency, with respect to PC1, which explains 99.4% of the variance. Separations between plantlike flavour, plantlike aroma, astringency, and acidity were also noted, as the astringency was on the top, left-hand side of the plot and acidity was on the bottom, left-hand side of the plot. This plot shows that that the formulations with low skin extract contents, the 0, 8, and 16% skin extract formulations, are more associated with plum flavour, plum aroma and sweetness, while the formulations containing higher percentages of skin extract, the 24 and 32% skin extract formulations, are associated with plantlike flavour, plantlike aroma, astringency and acidity. The increases in skin extract content in the formulations lead to an increase in the total polyphenolic content and antioxidant activity. This, in turn, caused an increase in astringency (Singleton & Noble, 1976).

Consumer liking

The consumer group (N=100) consisted of 67% female and 23% male consumers. Consumers that consumed fruit beverages seven times a week or more amounted to 26% of the total consumer group, while 42% of consumers consumed fruit beverages three times a week. The consumers that consumed fruit beverages about once a week amounted to 22%. Only 6% of the consumers consumed fruit beverages approximately twice a month, while the remaining 4% of consumers consumed fruit beverages less than once a month. This indicates that the largest part of the consumer group consumed fruit beverages three times a week or more.

The effect of consumer demographics can be determined by observing if the probability of significant ($P\leq0.05$) difference is visible for the chi-squared value (Lawless & Heymann, 1998). A significant ($P\leq0.05$) difference was detected for gender by consumption for the consumers ($\chi^2=21.01$; $P=0.099$). This means that the males and females do not consume fruit beverages

equally frequently. Females were found to consume fruit beverages less often than males (data not shown).

Preference

The degree of liking for the nectar formulations by the consumers (N=100) is illustrated in Table 10. There was no significant (P>0.05) difference in consumer preference for the 0 and 16% skin extract formulations. These formulations were most preferred by consumers. No significant (P>0.05) differences in preference were determined between the 8, 16 and 24% skin extract formulations. A significant (P>0.05) difference in preference was, however, detected between the 32% skin extract formulation and the other four formulations (P≤0.05). This formulation was least preferred by consumers. This is expected, as the 32% skin extract formulation had the greatest skin extract content, which presented a greater astringency than the other formulations due to its higher polyphenolic content (Brossaud *et al.*, 2001).

Acceptability

The frequency of the scores (Figure 4) indicate the part of the scale which was most used by the consumers to rate the plum nectar formulations. Figure 4 indicates the distribution of the scores over the nine categories of the nine-point hedonic scale (Lawless & Heymann, 1998) and thus, the acceptability of the formulations. All of the formulations were considered acceptable, as 83% of consumers rated the nectar formulations between *Like slightly* and *Like extremely*. The 0, 8, 16, and 24% skin extract formulations received high ratings between *Like slightly* and *Like extremely*, while the 32% skin extract formulation was given the lowest rating between *Like slightly* and *Like extremely*, and the highest score for *Dislike slightly.* These results indicate that the 32% skin extract formulation was less acceptable to consumers compared to the other four formulations. The formulations that were most liked contained the least amounts of skin extract. These formulations were most liked because the formulations possessed the highest plum flavour, plum aroma, and sweetness attributes compared to the high skin extract formulations, which possessed undesirable sensory attributes, such as plantlike aroma, plantlike flavour, astringency, and acidity.

Correlation of chemical, sensory and consumer data

The discriminant analysis (DA) conducted on the five nectar formulations (Figure 5) showed distinct groupings of the formulations when analysed with the chemical and sensory data. There was no overlapping between the five formulations, indicating that these formulations were not similar with respect to chemical or sensorial attributes. The F1 values of formulations followed a decreasing trend with an increase in skin extract content. The 24 and 32% skin extract formulations were grouped on the left-hand side of the plot, while the 16% skin extract formulation was found close to the centre of the plot. The 0 and 8% skin extract formulations were situated on

the right-hand side of the plot. In Figure 5, F1 and F2 explain 99.64% of the variance in the analysis.

The PCA loadings plot (Figure 6a) illustrated the association between the chemical attributes, sensory attributes and degree of liking of the plum nectar formulations. In these plots, PC1 and PC2 explain 99.08% of the variance. Figure 6 (a) illustrates that all the chemical attributes are associated with the sensory characteristics astringency, acidity, plantlike flavour, and plantlike aroma, with the exception of pH, which lies towards sweetness, plum flavour, and plum aroma. Liking was associated with sweetness, plum flavour, and plum aroma, indicating that consumers preferred the formulations with high levels of these attributes. Figure 6 (b) shows the placement of the five plum nectar formulations. Figure 6 (b) indicates that the 24 and 32% skin extract formulations were associated with the chemical and sensory characteristics (plantlike aroma, plantlike flavour, acidity, and astringency), which are similarly situated in Figure 6 (a). The 0, 8, and 16% skin extract formulations possessed the attributes equally situated in Figure 6 (a), namely sweetness, plum flavour, plum aroma, pH, and consumer liking. pH was associated with sweetness, plum flavour, and plum aroma, as the formulations possessing lower skin extract contents (0, 8, and 16% skin extract formulations) had higher pH values than the formulations with higher skin extract content (24 and 32% skin extract formulations). The alignments on these plots are due to higher concentrations of polyphenolic compounds and higher antioxidant activities, resulting from the increased skin extract content of the formulations.

CONCLUSION

Chemical analyses conducted on the 0, 8, 16, 24, and 32% skin extract formulations and twenty-two commercial red, violet, and blue fruit beverage samples indicated that the formulations fell within the ranges presented by the commercial beverages for colour, antioxidant activity, and polyphenolic assays. The 32% skin extract formulation had the highest polyphenolic content and antioxidant activity, indicating that the greatest amount of skin extract contributed the most polyphenolic compounds. Consumer preference of the formulations decreased with an increase in skin extract content. Plum aroma, plum flavour, and sweetness decreased with an increase in skin extract content. Astringency, acidity, plantlike flavour, and plantlike aroma increased with an increase in skin extract. This caused the formulations with high skin extract contents to be less acceptable to consumers. Astringency occurs with an increase in polyphenolic content, but polyphenolic compounds possess health-promoting properties. A balance between these compounds and consumer preference thus had to be found. Considering all these factors, the 24% skin extract formulation was an optimum formulation. This formulation contained relatively high amounts of polyphenolic compounds when compared to the 0, 8, and 16% skin extract formulations, but did not possess the undesirable sensory attributes of the 32% skin extract formulation.

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Table 1. List of chemicals and suppliers of the chemicals used in the chemical analyses of the commercial beverage and plum nectar samples

Chemical	Supplier
2,2'-Azo-bis-(2-methylpropionamidine) Dihydrochloride (AAPH), 97%	Aldrich; Steinheim, Germany
2,2-Diphenyl-1-picrylhydrazyl (DPPH)	Sigma, Sigma-Aldrich; Steinheim, Germany
2,4,6-Tri-(2-pyridyl)-*s*-triazin (TPTZ)	Fluka, Sigma-Aldrich; Steinheim, Germany
3,4,5-Trihydroxybenzoic acid (Gallic acid)	Sigma; Steinheim, Germany
6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)	Aldrich; Steinheim, Germany
Acetonitrile, LiChrosolv gradient grade for liquid chromatography	Merck; Darmstadt, Germany
100% Glacial acetic acid	Merck; Darmstadt, Germany
Anhydrous sodium carbonate	Saarchem, Merck; Gauteng South Africa
Ascorbic acid	Sigma, Sigma-Aldrich; Steinheim, Germany
(+)-Catechin	Sigma, Sigma-Aldrich; Steinheim, Germany
Neochlorogenic acid	Phytolab; Vestenbergsgreuth, Germany
Cyanidin-3-glucoside	Extrasynthese; Genay, France
Cyanidin-3-rutinoside	Extrasynthese; Genay, France
HCl, 37%	Saarchem Premium, Merck; Gauteng, South Africa
$FeCl_3$	Riedel-de Haën, Sigma-Aldrich; Germany
Fluorescein disodium	Riedel-de Haën, Sigma-Aldrich; Germany
Folin-Ciocalteau reagent	Merck; Darmstadt, Germany
Formic acid, 98%	BDH; London, England
Methanol	Merck; Gauteng, South Africa
Potassium dihydrogen phosphate	Merck; Darmstadt, Germany
Quercetin-3-rutinoside	Sigma, Sigma-Aldrich; Steinheim, Germany
Quercetin-3-glucoside	Fluka, Sigma-Aldrich; Steinheim, Germany

Table 2. Sample detail from the containers of commercial beverages made from red, blue and violet fruits

Code	Type of beverage	Volume (mL)	Packaging	Ingredients
CS1	Blueberry	350	Clear plastic bottle	Apple juice, blueberry juice, citric acid, natural flavourants, antifoaming agent
CS2	Cranberry and Strawberry	350	Clear plastic bottle	Apple juice, strawberry juice, cranberry juice, flavourant, aronia berry juice, ascorbic acid, malic acid, antifoaming agent
CS3	Ruby Grapefruit	275	Glass bottle	Carbonated purified water, sugar, ruby grape fruit juice, clouding agents, citric acid, nature identical and natural flavourants, natural colourants
CS4	Berry	1000	Clear plastic bottle	Youngberry juice, pear juice, blackcurrant juice, raspberry oil, citric acid, ascorbic acid
CS5	Cranberry	1000	Clear plastic bottle	Apple juice, cranberry juice, natural flavourants, aronia berry juice, ascorbic acid, antifoaming agent
CS6	Pomegranate	330	Can	Apple juice, pomegranate juice, aronia berry juice, lemon juice, natural flavourants, CO_2
CS7	Pomegranate	500	Clear plastic bottle	Pomegranate concentrate, natural flavourant, malic acid. Preservative: Natamax
CS8	Cranberry	500	Clear plastic bottle	Cranberry concentrate, natural flavourant. Preservative: Citric acid
CS9	Rooibos and Cranberry	500	Clear plastic bottle	Rooibos tea, apple &/or grape juice, cranberry juice, aronia berry juice, sucrose, fructose, citric acid, flavourants, pimaricin, sodium benzoate, potassium sorbate
CS10	Strawberry	200	Tetrapak	Grape &/or pear juice, strawberry pulp, vitamin A, vitamin E, ascorbic acid
CS11	Cranberry	200	Tetrapak	Grape juice, aronia berry juice, apple juice, cranberry juice, tartaric acid, vitamin A, vitamin E, ascorbic acid
CS12	Berry	200	Tetrapak	Youngberry juice, grape juice, blackcurrant juice, raspberry juice, natural flavourants, ascorbic acid
CS13	Cherries and Berries	200	Tetrapak	Grape &/or pear juice, apple juice, cherry juice, blackcurrant juice, vitamin A, vitamin E, ascorbic acid
CS14	Rooibos and Pomegranate	500	Clear plastic bottle	Rooibos tea, apple &/or grape juice, pomegranate juice, aronia berry juice, sucrose, fructose, pimaricin, sodium benzoate, potassium sorbate
CS15	Pomegranate	500	Clear plastic bottle	Deflavoured apple juice, pomegranate juice, acidity regulator, sodium benzoate, potassium sorbate, nature identical flavourants, stabiliser
CS16	Mixed Berry	350	Clear plastic bottle	Apple &/or grape juice, blackcurrant juice, passion fruit juice, strawberry juice, nature identical flavourants, colourants, sodium benzoate, potassium sorbate, pimaricin
CS17	Cranberry	1000	Tetrapak	Cranberry juice, apple juice, blended with grape &/or pear juice, nature identical flavourants
CS18	Cranberry and Cherry	1000	Tetrapak	Apple, grape or pear concentrate, cherry concentrate, cranberry concentrate, malic acid, natural and nature identical flavourants, natural colourants, aspartame, acesulfame K
CS19	Berry	330	Can	Water, apple juice, blackcurrant juice, grape juice, strawberry juice, raspberry juice, sugar, flavourants, ascorbic acid
CS20	Berry	1000	Tetrapak	Blackcurrant juice, strawberry juice, guava puree, raspberry juice, blended with grape &/or apple &/or pear juice, nature identical flavourants
CS21	Blackcurrant	500	Clear plastic bottle	Water, sucrose, natural tea extracts, citric acid, flavourants, sodium benzoate, pimaricin, sodium citrate, colourants, epigallocatechin gallate
CS22	Ruby Grapefruit	1000	Tetrapak	Ruby grapefruit juice, grape &/or pear juice, vitamins, nature identical flavourants

Table 3. Calculations for the preparation of the plum nectar formulations (example for 30g portion with 20% skin extract)

Source	Mass (g)	°Brix	Product = Mass x °Brix
Pulp	(30 x 40)/100 **= 12**	15	12 x 15 = 180
Skin extract	(30 x 20)/100 **= 6**	5	6 x 5 = 30
Concentrate	(unknown)	70	(unknown) x 70
Total Nectar	**30**	13	30 x 13 = 390
Concentrate calculation	(unknown) = [390 - (180 + 30)]/70 = **2.57**		
H_2O calculation	30 - (12 + 6 + 2.57) = **9.43**		

Table 4 Chemical attributes of concentrates acquired for product development of a functional plum nectar

Concentrate type	Date obtained	Provider	°Brix	pH	Titratable acidity
Apple	23/05/2009	Pacmar	70.00	3.27	15.34 g malic acid/L
Grape	03/06/2009	KWV	68.50	3.71	12.22 g tartaric acid/L
COT Filtered	03/06/2009	KWV	72.01	2.76	5.62 g tartaric acid/L
Grape	12/08/2009	KWV	68.48	3.69	14.74 g tartaric acid/L
Pear	05/06/2009	Appletiser	71.27	3.63	2.04 % malic acid (w/w)
Apple	04/06/2009	Distell	70.00	3.68	18.05 g malic acid/L

COT – colourless, odourless and tasteless concentrate

Table 5. HPLC gradients for determination of polyphenolic compound and ascorbic acid content in plum nectar formulations

Polyhenolic compounds[a]		Ascorbic acid[b]	
Time (min)	**% Solvent B**	**Time (min)**	**% Solvent B**
1	3	3	3
4	7	5	50
7	7	7	50
27	35	10	3
29	80	20	3
31	80		
35	3		
45	3		

[a] Solvent A = 7.5% formic acid in water, Solvent B = 7.5% formic acid in acetonitrile; [b] Solvent A = 0.1% formic acid in water, Solvent B = acetonitrile.

Table 6. Reference standards used in training of descriptive sensory panel

Reference	Specifications
Pulp	20 mL test batch pulp
Skin	20 mL test batch skin extract
Grape concentrate	20 mL KWV grape concentrate diluted to 13 °Brix
Plum jam	10 g Bloublommetjieskloof plum jam
Prune juice	20 mL SAD prune juice
Prune	1 SAD prune
Alum	20 mL of a 10% solution
Citric acid	20 mL of a 10% solution

SAD – South African Dried Fruit Co-operation; KWV - Koöperatieve Wijnbouwers Vereniging, South Africa.

Table 7. The chemical analyses conducted on commercial red, blue and violet fruit beverages

Sample	TSS	pH	TA	Colour		ORAC		DPPH		FRAP		Total Phenols		Total Anthos	Ascorbic Acid
				Total	Red (520 nm)	Original	Adjusted	Original	Adjusted	Original	Adjusted	Original	Adjusted		
CS 1	10.7	3.29	10.89	19.73	0.92	12106.5	12106.5	1589.2	1589.2	72.86	72.86	438.11	438.11	34.17	ND
CS 2	10.9	3.44	7.77	7.07	0.31	10353.8	10209.9	3156.6	2525.6	411.32	221.12	547.91	526.06	17.62	157.52
CS 3	11.4	2.69	7.93	4.60	0.16	2281.9	2281.9	211.3	211.3	8.07	8.07	53.97	53.97	3.74	ND
CS 4	15.3	3.48	13.85	18.70	0.89	23016.9	22928.8	4932.8	4546.6	405.54	289.11	875.36	861.98	44.34	96.42
CS 5	11.3	3.39	8.22	4.96	0.18	10436.1	10049.1	6167.2	4470.0	750.36	238.81	788.79	730.02	6.53	423.64
CS 6	11.3	3.40	12.65	8.89	0.33	9071.7	9071.7	2673.7	2673.7	103.13	103.13	441.35	441.35	12.27	ND
CS 7	10.3	3.24	8.41	8.89	0.30	11819.3	11815.6	1698.2	1681.8	85.17	80.25	527.35	526.79	3.25	4.07
CS 8	11.0	3.14	10.56	7.87	0.25	19840.2	19835.9	2543.9	2525.3	103.03	97.42	803.58	802.93	6.48	4.65
CS 9	8.6	3.01	3.58	5.01	0.13	5195.9	5195.9	562.6	562.6	47.92	47.92	149.19	149.19	4.31	ND
CS 10	12.7	3.46	5.24	6.54	0.19	7789.8	7304.2	5316.4	3186.8	876.46	234.55	916.48	842.74	7.38	531.59
CS 11	12.9	2.95	12.81	10.16	0.54	9508.5	9508.5	1215.5	1215.5	80.63	80.63	288.21	288.21	15.91	ND
CS 12	13.1	3.39	13.76	10.28	0.33	16799.9	16572.8	6513.9	5517.7	629.73	329.52	393.24	358.75	15.96	248.61
CS 13	12.9	3.39	5.05	7.17	0.23	5927.8	5927.8	827.6	827.6	101.55	101.55	668.01	668.01	5.60	ND
CS14	8.8	3.10	3.33	7.30	0.26	5574.9	5574.9	710.8	710.8	43.38	43.38	239.29	239.29	8.88	ND
CS 15	12.4	3.57	7.64	16.52	0.90	14937.9	14937.9	2282.2	2282.2	422.00	422.00	271.34	271.34	40.79	ND
CS 16	11.7	3.61	6.07	6.10	0.25	8294.7	8294.7	625.8	625.8	63.14	63.14	581.61	581.61	6.11	ND
CS 17	13.1	3.38	9.49	7.18	0.25	13303.7	13211.6	3218.5	2814.3	444.55	322.73	190.75	176.76	9.29	100.89
CS 18	8.8	3.27	7.42	5.98	0.20	8758.7	8758.7	1156.1	1156.1	79.17	79.17	348.47	348.47	5.58	ND
CS 19	11.2	3.05	8.19	6.58	0.23	13716.6	13664.6	2022.1	1794.1	328.21	259.51	352.46	344.56	5.60	56.90
CS 20	12.8	2.95	6.29	6.08	0.20	6428.9	6428.9	3242.4	3242.4	559.82	559.82	312.25	312.25	6.99	ND
CS 21	8.3	3.41	2.99	7.16	0.24	2237.6	2237.6	319.7	319.7	8.10	8.10	55.92	55.92	4.17	ND
CS 22	12.7	3.21	18.40	14.04	0.32	21922.5	21639.1	2631.9	1389.1	580.22	205.62	519.44	476.40	8.44	310.22
Minimum	8.3	2.69	2.99	4.60	0.13	2237.6	2237.6	21.1	211.3	282.02	8.07	53.97	53.97	3.25	ND
Maximum	15.3	3.61	18.40	19.73	0.92	23016.9	22928.0	2437.2	5517.9	875.82	559.82	916.48	861.98	44.34	423.64
Average	11.5	3.27	8.66	8.95	0.35	10878.4	810798.0	650.3	2084.9	281.91	175.84	443.77	431.58	12.42	193.45

Total soluble solids (TSS) - °Brix; Titratable acidity (TA) – g/L; Colour – Absorbance; ORAC – µmol Trolox equivalents/L; DPPH - µmol Trolox equivalents/L; FRAP - µmol Trolox equivalents/L; Total phenols - mg gallic acid equivalents/L; Total anthocyanins - mg cy-3-glc/L; AA – g/L; Original – results of beverages as determined; Adjusted – results adjusted by subtracting the ascorbic acid contribution.

Table 8. The physicochemical characteristics of plum pulp and skin extract (2009 harvest)

	TSS	Titratable Acidity	pH
Test batch pulp	18.48	5.39	3.15
PR05-17 pulp	14.45	5.24	3.30
PR05-17 skin extract	5.30	18.26	3.71

Total soluble solids (TSS) - °Brix; Titratable acidity (TA) – g malic acid/L.

Table 9. Acidity, colour, antioxidant activity, and polyphenolic content attributes of red-fleshed plum nectar formulations

Samples	TA	pH	Colour		ORAC	DPPH	FRAP	Total Phenols	Total Anthos	HPLC					
			Total	Red (520 nm)						Cy-3-glc	Cy-3-rut	Q-3-rut	Q-3-glc	Q-3-xyl	NeoChA
0% skin extract	10.12	3.33	12.68^{c}	0.48^{c}	18597.1^{c}	2143.4^{bc}	319.2^{c}	436.7^{c}	14.34^{c}	9.88^{e}	15.72^{e}	4.37^{e}	2.39^{e}	1.31^{e}	4.17^{c}
8% skin extract	10.37	3.32	12.96^{bc}	0.51^{c}	19315.5^{abc}	2196.9^{c}	325.1^{c}	482.3^{b}	16.19^{c}	10.99^{d}	17.02^{d}	5.23^{d}	3.19^{d}	2.40^{d}	4.23^{bc}
16% skin extract	10.26	3.32	13.44^{bc}	0.54^{bc}	19586.1^{bc}	2392.1^{bc}	338.9^{bc}	473.8^{b}	18.23^{b}	12.06^{c}	18.28^{c}	6.03^{c}	3.94^{c}	3.33^{c}	4.27^{b}
24% skin extract	10.42	3.32	14.60^{ab}	0.59^{ab}	19830.1^{ab}	2410.5^{b}	351.6^{ab}	512.9^{ab}	18.12^{b}	12.92^{b}	19.17^{b}	6.77^{b}	4.75^{b}	4.42^{b}	4.28^{bc}
32% skin extract	10.84	3.3	17.17^{a}	0.69^{a}	20734.6^{a}	2547.5^{a}	372.8^{a}	536.1^{a}	19.64^{a}	14.18^{a}	20.72^{a}	7.62^{a}	5.59^{a}	5.42^{a}	4.39^{a}
LSD (P = 0.05)	NA	NA	1.71	0.06	2267.2	16.7	5.3	1.9	32.43	0.58	0.89	0.49	0.24	0.31	0.11

Titratable acidity (TA) – g malic acid/L; Total phenols - mg gallic acid equivalents/L; ORAC - µmol Trolox equivalents/L; DPPH - µmol Trolox equivalents/L; FRAP - µmol Trolox equivalents/L; Total Anthos (Total anthocyanins) - mg cy-3-glc/L; Ascorbic acid - mg/L; HPLC – mg/L; Cy-3-glc – cyanidin-3-glucoside; Cy-3-rut – cyanidin-3-rutinoside; Q-3-rut – quercetin-3-rutinoside; Q-3-glc – quercetin-3-glucoside; Q-3-xyl – quercetin-3-xyloside; NeoChA – neochlorogenic acid; LSD – Least significant difference at the 5% level of significance, means with different superscripts differ significantly from each other at the 5% significance level. NA – significant difference not analysed statistically.

Table 10. Preference for the plum nectar samples for the total group of consumers (N = 100)

Treatment formulations	Mean score for degree of liking
0% skin extract	7.2[a]
8% skin extract	6.9[b]
16% skin extract	7.0[ab]
24% skin extract	6.7[b]
32% skin extract	5.8[c]
LSD (P = 0.05)	0.33

LSD = Least significant difference at the 5% level of significance. Means with different superscripts differ significantly from each other at the 5% significance level.

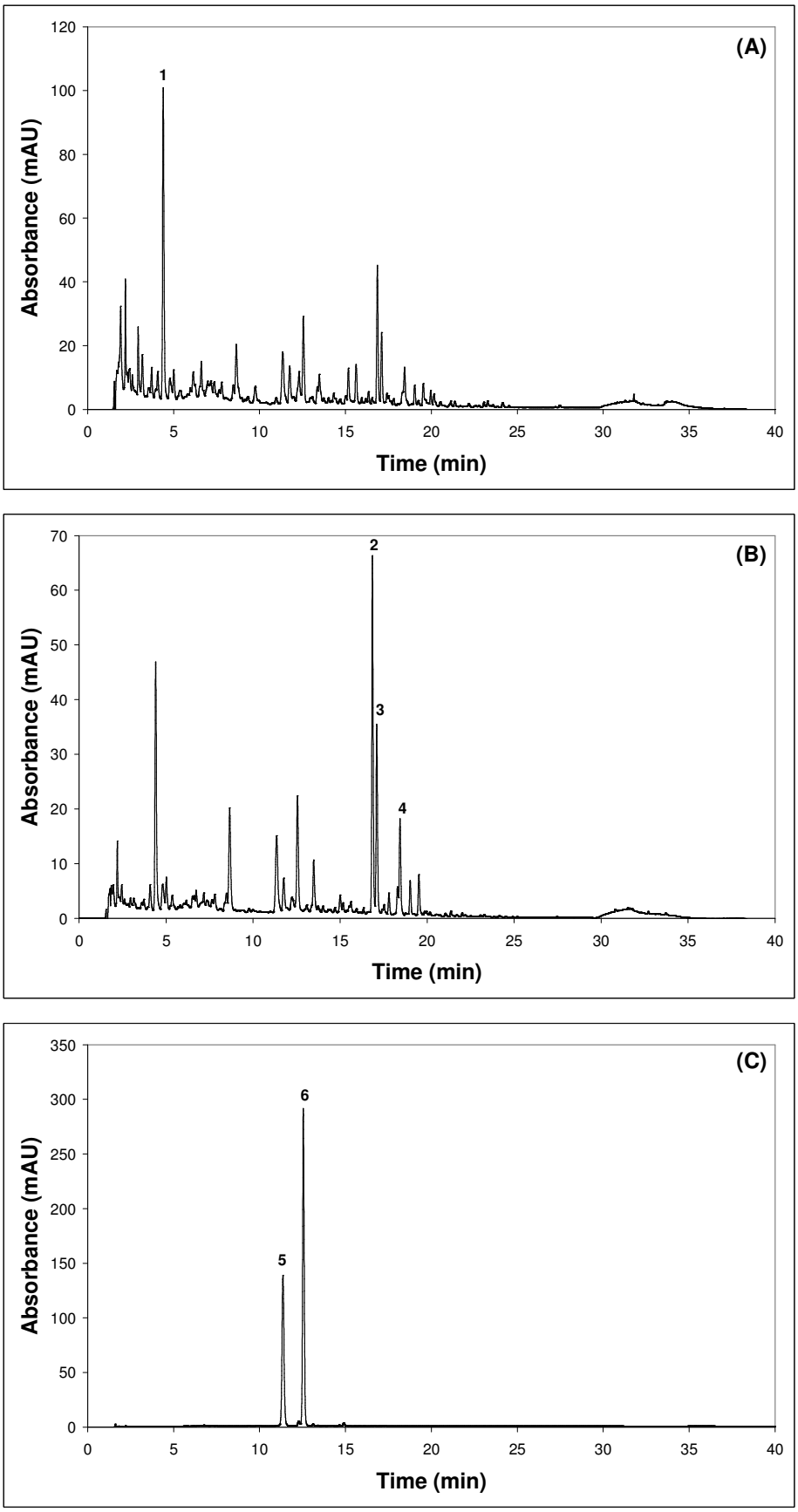

Figure 1 Example of the HPLC chromatograms for a 0% skin extract plum nectar formulation at **(A)** 320 nm, **(B)** 350 nm and **(C)** 520 nm (**1** - neochlorogenic acid, **2** - quercetin-3-rutinoside, **3** – quercetin-3-glucoside, **4** – quercetin-3-xyloside, **5** – cyanidin-3-glucoside, **6** – cyanidin-3-rutinoside).

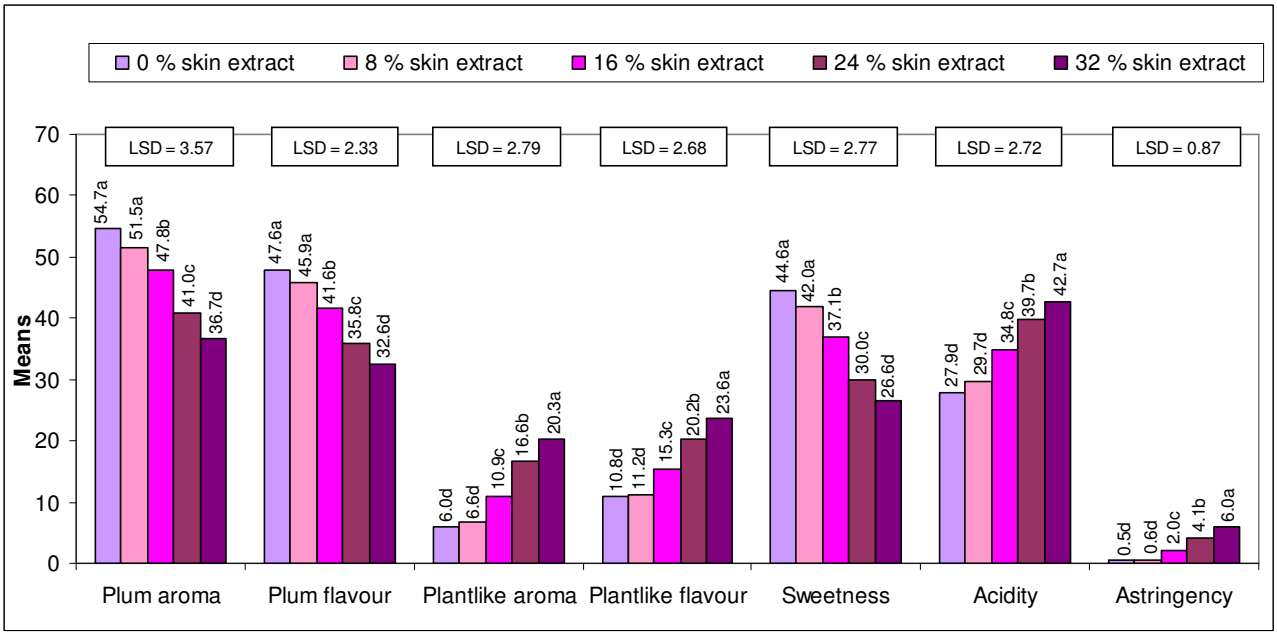

Figure 2 Sensory attributes for the five plum nectar formulation samples tested by trained panel. LSD determined at the 5% level.

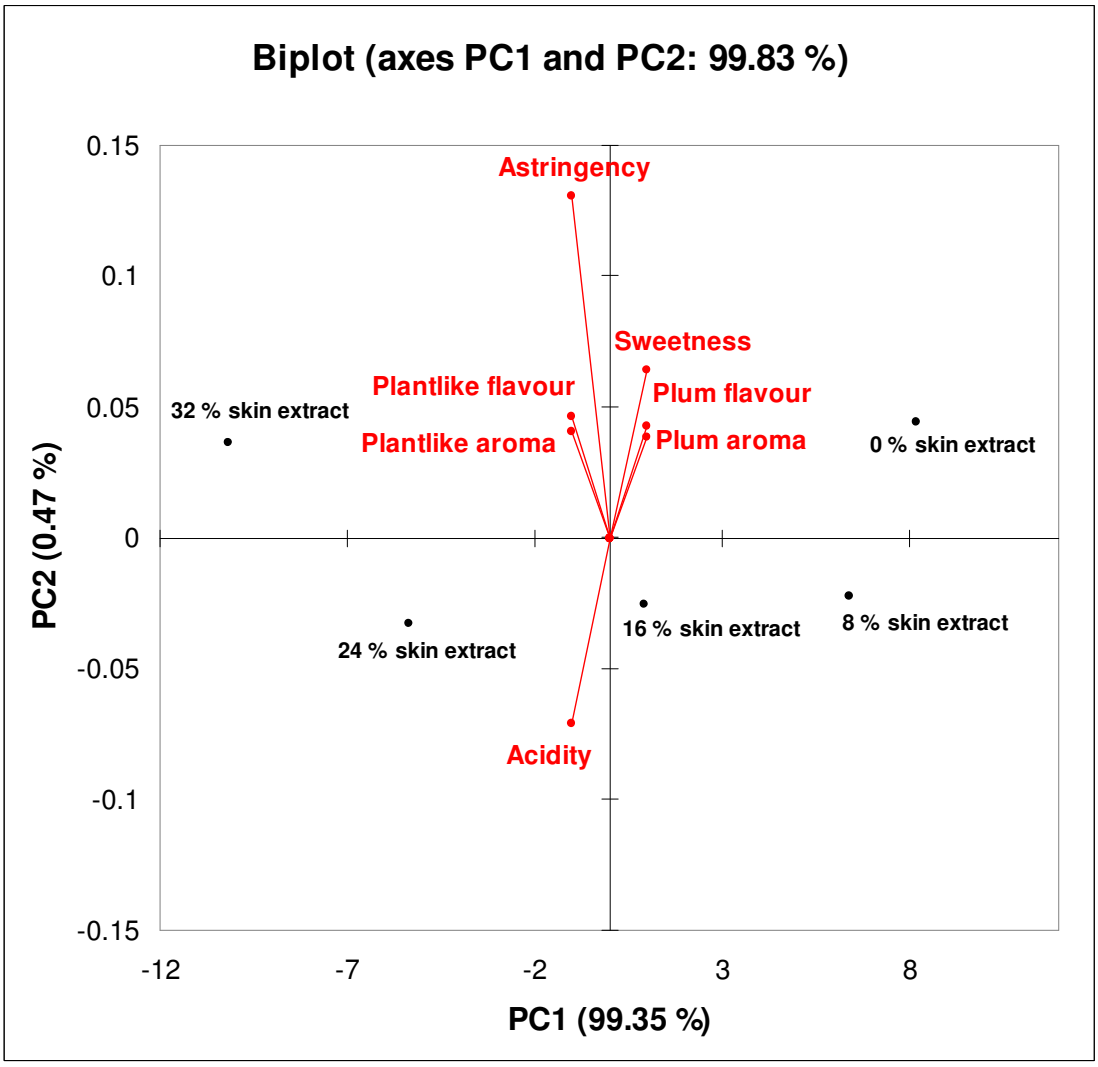

Figure 3 PCA bi-plot of the descriptive sensory attributes (loadings; red) given for the plum nectar formulations containing 0, 6, 18, 24, and 32% skin extract (scores; black).

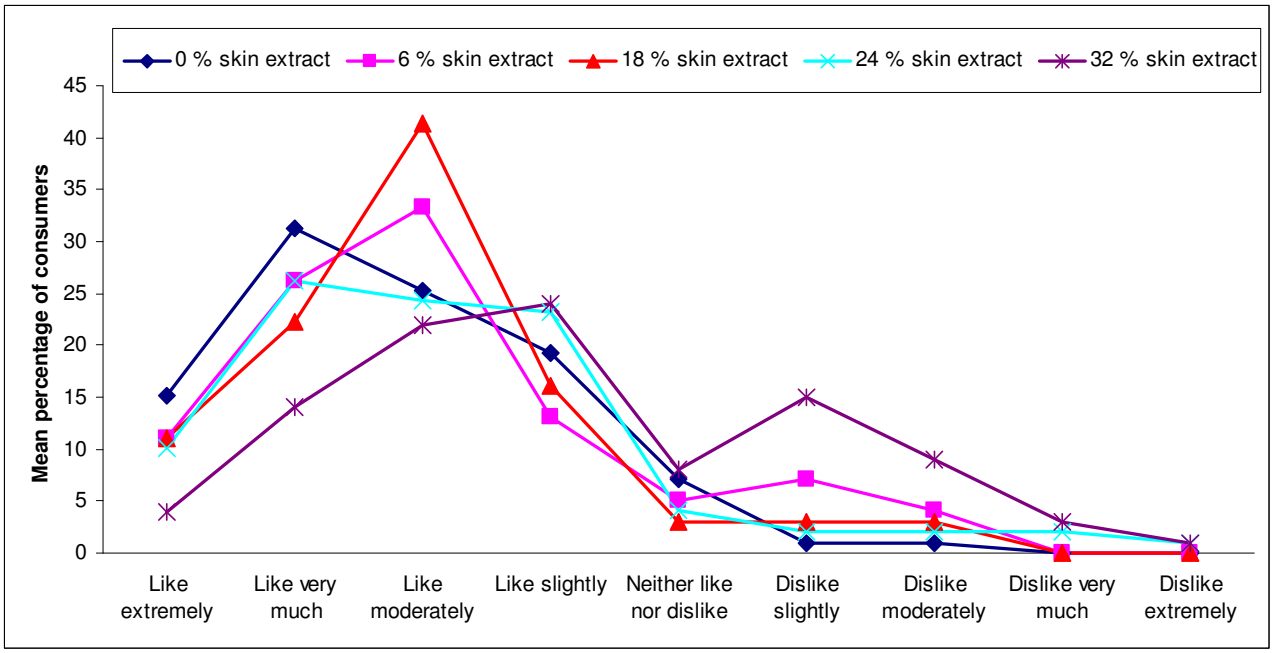

Figure 4 Distribution of scores for the five plum nectar formulations (0, 8, 16, 24, and 32% skin extract formulations) ranked by the total group of consumers (N=100) with respect to degree of liking (χ^2 = 42.644; P = 0.099).

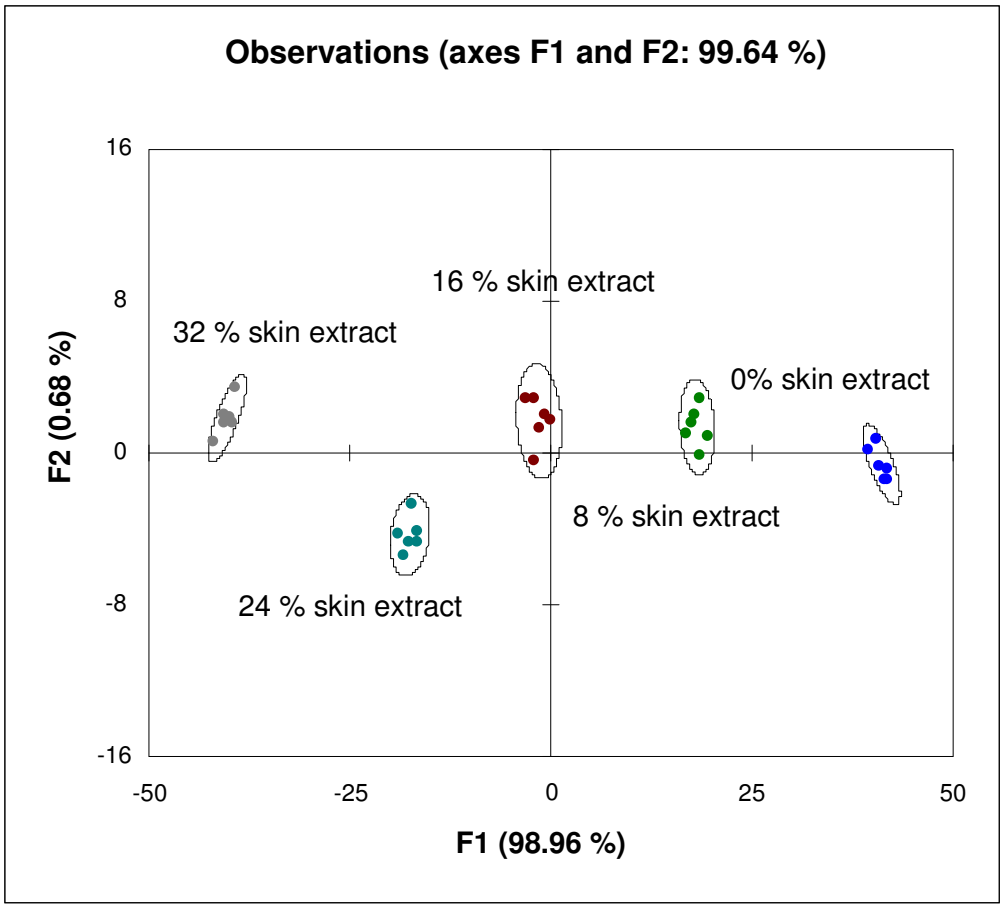

Figure 5 Discriminant analysis (DA) conducted on the five plum nectar formulations (0, 8, 16, 24 and 32% skin extract formulations) with respect to the chemical and sensory attributes.

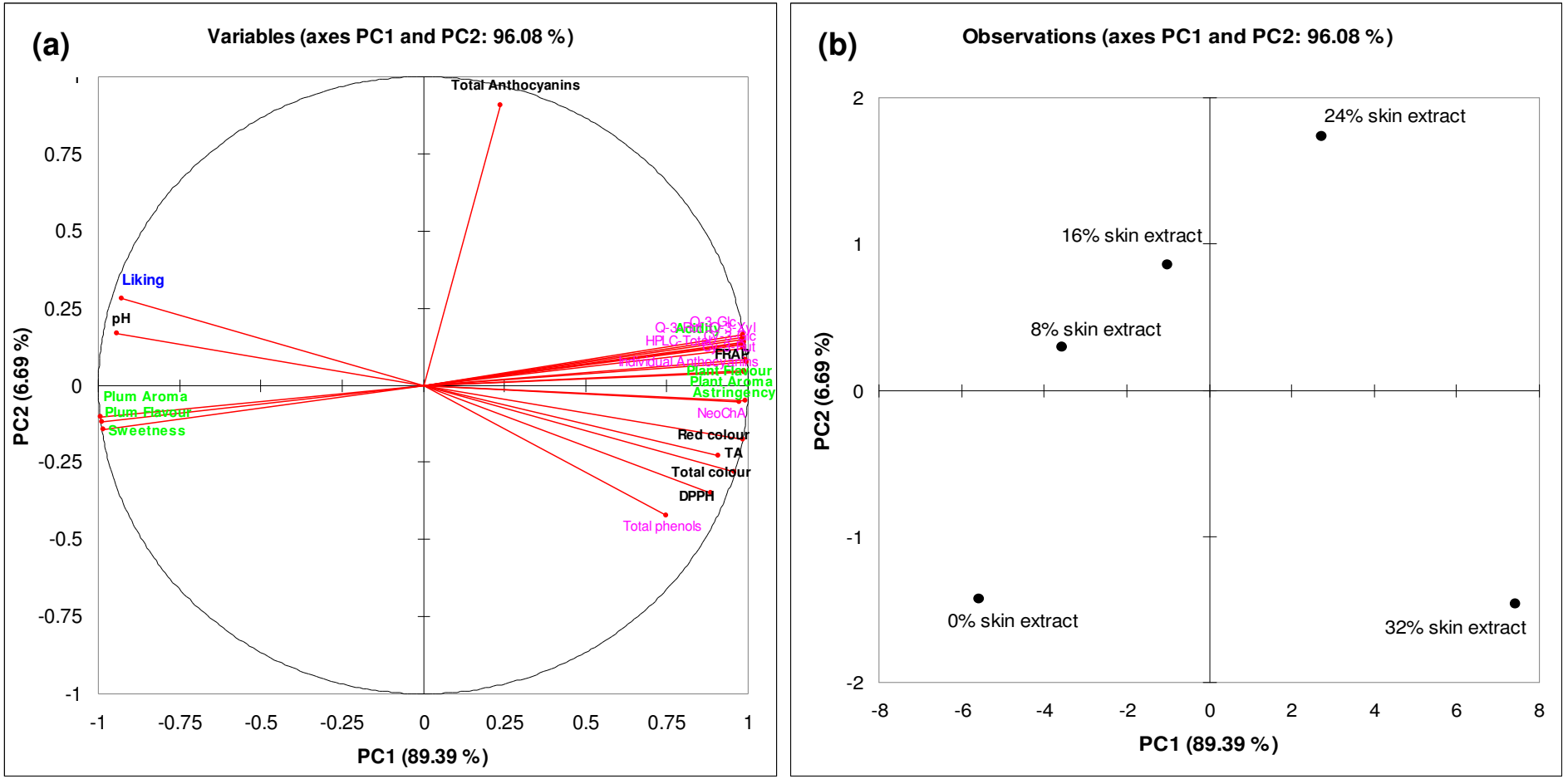

Figure 6 (a) PCA loadings plot of the chemical attributes (in red and black), sensory attributes (in green), and degree of liking (in blue). **(b)** The PCA scores plot of the five skin extract formulations. PC1 and PC2 explain 96.08% of the variance.

Chapter 4

The effect of storage at shelf-life conditions on the quality attributes of red-fleshed Japanese plum nectar formulations

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ABSTRACT

A shelf-life study was conducted on plum nectar formulations in two phases. Phase 1 of the shelf-life study was conducted to determine the effects of storage on the chemical attributes of the plum nectar formulations after 24 weeks, while Phase 2 of the shelf-life study was conducted to determine whether any sensory changes occurred during 3 months of storage. For Phase 1 of the shelf-life study, the 0, 16, and 24% skin extract formulations were prepared in triplicate and stored at 0 and 5°C. The time points of this study were: before pasteurisation, after pasteurisation (week 0), and after 1, 2, 4, 6, 8, 12, 16, 18, 20, and 24 weeks of storage. Phase 2 of the shelf-life study was only conducted on the 0 and 24% skin extract formulations stored at 5°C, with sampling done at 0, 1, 2, and 3 months. The chemical attributes measured for the formulations of Phase 1 and Phase 2 of the shelf-life study at each time point included total colour, red colour, DPPH$^{\bullet}$ scavenging activity, total polyphenolic content, total anthocyanin content, and individual phenolic compound content. Significant decreases in cyanidin-3-glucoside, cyanidin-3-rutinoside and total anthocyanin contents were observed after 12 weeks of storage in both Phase 1 and Phase 2 of the shelf-life study for all formulations and storage temperatures. Between 12 and 24 weeks of storage, the degradation of these compounds slowed. The quercetin-3-glucoside and quercetin-3-xyloside contents of all formulations decreased significantly ($P \leq 0.05$) over 24 weeks in Phase 1 only at 0°C, while no change was observed over 3 months in Phase 2. Total and red colour only decreased significantly ($P \leq 0.05$) for some formulation x time combinations during Phase 1, with no change observed during Phase 2. The remaining chemical attributes of the formulations were stable over time in both Phase 1 and Phase 2 of the shelf-life study. Analyses of the slopes of the regression of the results from Phase 1 indicate that the decrease in chemical attribute values were mostly similar for different formulations at the same temperature. Differences in slopes between storage temperatures were not uniform, with total and red colour showing a faster rate of decrease at 5°C compared to 0°C, while the opposite was observed for quercetin-3-glucoside and quercetin-3-xyloside content.

Results from a PCA analysis of the chemical attributes from Phase 1 further indicated a close association between the 16 and 24% skin extract formulations, as well as between these formulations and all the chemical attributes. The sensory characteristics for the 0 and 24% skin extract formulations included plum flavour, plantlike flavour, raisin flavour, sweetness, acidity, and astringency. These sensory attributes of the formulations remained unchanged during storage. The results from Phase 1

and Phase 2 of the shelf-life study indicate that the formulations could be beneficial to the juice industry, as they are chemically stable after 24 weeks of storage, with the exception of the anthocyanins, and do not undergo noteworthy changes in taste, flavour and mouthfeel attributes during at least 3 months.

KEYWORDS Anthocyanins, chemical analyses, plum nectar formulation, *Prunus salicina,* multivariate analysis, sensory analysis, shelf-life, total polyphenolic content.

INTRODUCTION

The shelf-life stability of a product is affected by various intrinsic factors, such as the composition and pH of the product (Wang *et al*., 1995). Extrinsic factors, such as storage time, storage temperature, and light also affect the shelf-life stability of a product. This is especially true for products with high polyphenolic content. An increase in pH can lead to a decrease in anthocyanin stability (Cabrita *et al*., 2000), while high temperatures can adversely affect the stability of polyphenolic compounds by increasing the rate of degradation reactions (Piga *et al*., 2003). The presence of light and certain degrading enzymes or complexing compounds also reportedly degrade anthocyanins (Will & Dietrich, 2006), whereas transition metals, such as copper ions, can assist the degradation of anthocyanins (García-Viguera & Bridle, 1999). A decrease in the antioxidant activity of anthocyanin-containing juices has also been noted in the presence of ascorbic acid (González-Molina *et al*., 2009).

The influence of storage conditions was noted in a shelf-life study conducted by Kalt *et al*. (1999). This study indicated that storage conditions affected the total anthocyanin and polyphenolic contents of fruits, such as blueberries, strawberries and raspberries. During storage, anthocyanins degrade to colourless compounds or undergo polymerisation (Wang *et al*., 1995). In Japanese plums, the loss of antioxidant capacity is due to the oxidative degradation of polyphenolic compounds (Piga *et al*., 2003). These oxidative reactions result in nutritive losses, as well as changes in texture, aroma, and taste (Duda-Chodak & Tarko, 2007). Plum pastes, prepared from dark Stanley plums (25 °Brix), presented no significant ($P>0.05$) decrease in colour, acidity, consistency and flavour during storage at 4°C for 24 weeks. The plum pastes, however, presented significant ($P\leq0.05$) changes in sweetness and overall consumer acceptance during storage (Wang *et al*., 1995). The latter results pose the question as to whether the sensory attributes of nectar formulations produced from a fruit genotype with a high antioxidant capacity will be stable during storage. In view of this, this study focused on the chemical and sensory shelf-life stability of plum nectar formulations in two phases.

Phase 1 of the shelf-life study was conducted to determine whether 24 weeks of storage at 0 and 5°C influenced the colour, antioxidant activity, and polyphenolic content of the plum nectar formulations, each containing 0, 16, and 24% skin extract, respectively. The storage temperatures of 0

and 5°C were representative of possible commercial storage conditions. The formulations for Phase 1 were chosen by taking into account differences observed between formulations in Chapter 3. The 32% skin extract formulation was omitted, as it was significantly ($P \leq 0.05$) less preferred by consumers, while the 8% skin extract formulation was omitted due to the similarity of its chemical and sensory attributes with those of the 0 and 16% skin extract formulations. Phase 2 of the shelf-life study was conducted to determine the effect of storage on the sensory characteristics of the 0 and 24% skin extract formulations over a 3-month storage period at 5°C. Formulations prepared in Phase 2 were also analysed chemically to validate the results from Phase 1, as plums from a different harvest season were used.

MATERIALS AND METHODS

Chemicals

The chemicals used and their suppliers are shown in Table 1.

Fruit processing

A batch of fruit from the red-fleshed Japanese plum selection (*Prunus salicina* L.), PR05-17 (23.7 kg), was obtained from Bienne Donné farm (Groot Drakenstein, South Africa; S 33.84303, E 18.98039) in January 2010. This batch of plums was processed into plum pulp (10.5 kg) and skins (3.4 kg) as described in Chapter 3. A skin extract was prepared as described in Chapter 3. The total soluble solids content and pH of the plum pulp and skin extract was determined as described in Chapter 3 (Table 2).

Shelf-life study of nectar formulations

Plum formulations were prepared, as described in Chapter 3, using plum pulp, skin extract and grape concentrate (KWV, South Africa). Aliquots of each sample from Phase 1 and Phase 2 of the shelf-life study were collected in plastic microfuge tubes (2 mL) and stored in a freezer (-15°C) until chemical analyses could be performed. The temperatures of the 0 and 5°C storage rooms were monitored using an AZ 8835 T.RH% Logger Datalogger (Taichung City, Taiwan) during storage.

Phase 1

Plum pulp and skins from the 2009 harvest (Chapter 3) were used during Phase 1 of the shelf-life study. Phase 1 of the shelf-life study was conducted over 24 weeks on the 0, 16, and 24% skin extract formulations. Triplicates of each formulation were prepared, decanted into twenty-two glass vials (20 mL) and sealed with aluminium crimp caps with butyl septa. The vials were pasteurised in a water bath (93°C; 40 min). The internal temperature of the nectar formulations reached 90°C in ca 30 min.

Half of the vials were stored at 5°C and the rest at 0°C. The time points for sampling were: before pasteurisation, after pasteurisation (week 0), and after 1, 2, 4, 6, 8, 12, 16, 18, 20, and 24 weeks of storage. Samples were analysed for 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, total polyphenolic content, spectrophotometric total anthocyanin content, and individual phenolic compound content (HPLC), as described in Chapter 3.

Phase 2

The 0 and 24% skin extract formulations were prepared using pulp and skin extract from fruits harvested in 2010, decanted into 500 mL glass bottles, and sealed with a screw-cap before undergoing pasteurisation in a water bath (93°C; 40 min). The internal temperature of the nectar formulations reached ± 80°C in ca 30 min. The formulations were prepared once a month for three months (preparation time points: 0, 1, 2, and 3 months) and stored at 5°C in order to test all the formulation x time combinations simultaneously.

Sensory analysis, using an eight-member panel of judges, was conducted on the plum nectar formulations to determine the effects of storage on the sensory attributes of the nectars. As explained in Chapter 3 (Lawless & Heymann, 1998), the sensory attributes were measured on a line scale ranging from *None* to *Prominent* (0 - 100) and included plum flavour, plantlike flavour, raisin flavour, astringency, acidity, and sweetness. The reference standards used during the training of the panel are shown in Table 3. The questionnaire is attached as Addendum 3. Samples were also analysed for colour, $DPPH^{\bullet}$ scavenging activity, total polyphenolic content, total anthocyanin content, and individual phenolic compound content (HPLC), as described in Chapter 3.

Statistical analyses of data

The experimental design for Phase 1 of the shelf-life study was a randomised complete block design with three formulations x two storage temperatures x twelve time points. The experimental design for Phase 2 of the shelf-life study was a randomised complete block design with two formulations x four time points with four replications of each treatment combination. Multivariate statistical analysis was performed on the data from Phase 1 using the XLSTAT software (Version 7.5.2, Addinsoft, New York, USA). Principle component analysis (PCA) was conducted on the data from Phase 1 to test for an association between the attributes and the treatments. Regression analysis was also conducted on the data from Phase 1 to determine whether the attributes changed significantly ($P \leq 0.05$) over time. The before pasteurisation time point measurements were not included for the chemical analysis of Phase 1. Analysis of variance (ANOVA) using SAS® (Version 9; SAS® Institute Inc, Cary, USA) was conducted on the slopes of the regression of the data from Phase 1 to determine whether the chemical attributes increased or decreased during storage and to what extent this occurred. The data obtained from both Phase 1 and Phase 2 of the shelf-life study were analysed using ANOVA, along

with a student's t-test, indicating a least significant difference (LSD) at the 5% significance level. The Shapiro-Wilk test was implemented on these data to test the level of normality in the data (Shapiro & Wilk, 1965).

RESULTS AND DISCUSSION

Phase 1

Phase 1 of the shelf-life study was performed to determine the effect of storage time and temperature on the colour, antioxidant activity, and polyphenolic content of the formulations. The average temperatures of the cold storage rooms where the formulations were stored were monitored for fluctuations in temperature that could adversely influence the results of Phase 1. The average temperature of the storage rooms, designated as 0°C and 5°C, were 0.9°C and 4.7°C, respectively (data not shown).

The regression analysis of the chemical attributes for the 0, 16, and 24% skin extract formulations over time are represented in Table 4. P-values, in bold, indicate which of the formulation x temperature combinations of each attribute show a significant ($P \leq 0.05$) trend. The R^2 for red colour, total anthocyanins, cyanidin-3-glucoside, and cyanidin-3-rutinoside was significant ($P \leq 0.05$) for all the formulations at both 0 and 5°C over time (Table 4). This indicated that these attributes were not stable during 24 weeks of storage. Quercetin-3-glucoside and quercetin-3-xyloside also presented significant ($P \leq 0.05$) R^2-values for the formulations stored at 0°C, but not at 5°C. These results indicated that temperature might have played a role in the degradation of these compounds during storage. Significant ($P \leq 0.05$) R^2 values for the total colour of all the formulations stored at 5°C, as well as for the 24% skin extract formulation stored at 0°C, were also observed, indicating the influence of storage temperature (Table 4). No significant ($P > 0.05$) trends were found for any of the formulation x temperature combinations over time in terms of total polyphenolic content, quercetin-3-rutinoside, or neochlorogenic acid. This indicates that these attributes were relatively stable over time for all the formulations during storage at 0 and 5°C. $DPPH^{\bullet}$ scavenging activity only showed a significant ($P \leq 0.05$) trend for the 0% skin extract formulation stored at 5°C (Table 4), and is therefore also considered relatively stable during storage.

An ANOVA (Table 5) was conducted to ascertain whether the regression slopes of the treatment combinations differed significantly ($P \leq 0.05$). All slopes for regressions with significant ($P \leq 0.05$) R^2 values were negative, indicating a decreasing trend for the respective attributes with increasing storage times. With regard to total colour (Table 5), among the significant ($P \leq 0.05$) regressions, the slopes for the 0 and 24% skin extract formulations stored at 5°C were significantly ($P \leq 0.05$) more negative than those of the other formulation x time combinations. The extent of the change can be seen in Table 6, showing a decrease in total colour of 33%, 18% and 16%,

respectively, over 24 weeks for the 0, 16, and 24% skin extract formulations at 5℃. The 24% skin extract formulation stored at 0℃ had the least negative slope, compared to the other formulation x time combinations. The total colour decreased significantly (P≤0.05) faster over 24 weeks for the 24% skin extract formulation at 5℃ than at 0℃. The total colour of the 24% skin extract formulation decreased with 6 and 16% over 24 weeks at 0 and 5℃, respectively (Table 6).

In terms of red colour (Table 5 & 6), the slopes for the 0 and 16% skin extract formulations at 0℃ were the least negative (8 and 4% decrease, respectively, over 24 weeks), with the slopes for all the formulations stored at 5℃ being the most negative (22 - 33% decrease over 24 weeks). The slope for 24% skin extract formulation stored at 0℃ was intermediate (16% decrease over 24 weeks), but still significantly (P≤0.05) different to the other formulation x time combinations. At 0℃, the red colour decreased faster for the 24% skin extract formulation compared to the other formulations, while the decrease was similar for all formulations stored at 5℃. For all formulations, the red colour decreased significantly (P≤0.05) faster at 5℃ compared to 0℃.

With respect to total anthocyanin content (Table 5 & 6), the slope for the 0% skin extract formulation stored at 0℃ was least negative (16% decrease over 24 weeks) decrease and did not differ significantly (P>0.05) from the slopes of the other formulation x time combinations, except for the 0% skin extract formulation stored at 5℃, which was most negative (25% decrease over 24 weeks). Thus, temperature affected the rate of total anthocyanin content decrease only for the 0% skin extract formulation. Very little significant differences were observed between formulation x temperature combinations in terms of the individual anthocyanin content. The slopes for the 0% skin extract formulation stored at 5℃ were the least negative for cyanidin-3-glucoside and cyanidin-3-rutinoside, but these formulations presented a great decrease in 24 weeks (50 and 36%, respectively). The most negative slopes were that of the 16% skin extract formulations stored at 5℃ for cyanidin-3-glucoside and cyanidin-3-rutinoside (44 and 39% decrease, respectively, over 24 weeks). In terms of temperature effects, only the 16% skin extract formulation showed a significantly (P≤0.05) higher rate of degradation for cyanidin-3-glucoside content at 5℃ compared to 0℃.

Only the decrease in quercetin-3-glucoside and quercetin-3-xyloside content at 0℃ will be discussed as regressions were not significant (P>0.05) at 5℃ (Table 5). In terms of quercetin-3-glucoside content, the slope for the 24% skin extract formulation was significantly (P≤0.05) more negative than that for the 0% skin extract formulation (12 and 15% decrease, respectively, over 24 weeks). The slope for the 16% skin extract formulation (10% decrease over 24 weeks) did not different significantly (P>0.05) from the 0 or 24% skin extract formulations. In terms of quercetin-3-xyloside, significantly (P≤0.05) more degradation occurred for the 16 and 24% skin extract formulation than for the 0% skin extract formulation (17, 19, and 22% decrease for the 0, 16 and 24% skin extract formulations, respectively, over 24 weeks).

In the PCA scores and loadings plots of the chemical results of Phase 1 of the shelf-life study (Figure 1), PC1 and PC2 explain 77% of the variance. The chemical attributes include, cyanidin-3-glucoside, cyanidin-3-rutinoside, quercetin-3-glucoside, quercetin-3-rutinoside, quercetin-3-xyloside, neochlorogenic acid, DPPH$^{\bullet}$ scavenging activity, total anthocyanin content, and total polyphenolic content. The total and red colour data were not analysed with the other chemical attributes in the PCA, because the colour data followed very different trends compared to the other chemical attributes. This caused a drastic change in the PCA plot, which was difficult to explain.

The flavonols, quercetin-3-glucoside, quercetin-3-rutinoside, and quercetin-3-xyloside are closely associated with each other (Figure 1 a). Close associations exist between quercetin-3-glucoside and quercetin-3-rutinoside ($r=0.984$; $P\leq0.05$), quercetin-3-glucoside and quercetin-3-xyloside ($r=0.996$; $P\leq0.05$), and quercetin-3-rutinoside and quercetin-3-xyloside ($r=0.973$; $P\leq0.05$). Similarly, the anthocyanins, cyanidin-3-glucoside and cyanidin-3-rutinoside, were closely associated ($r=0.993$; $P\leq0.05$) with each other (Figure 1 a). The total anthocyanin content was also positively associated with cyanidin-3-glucoside ($r=0.846$; $P\leq0.05$), cyanidin-3-rutinoside ($r=0.812$; $P\leq0.05$), quercetin-3-glucoside ($r=0.793$; $P\leq0.05$), quercetin-3-rutinoside ($r=0.742$; $P\leq0.05$), and quercetin-3-xyloside ($r=0.796$; $P\leq0.05$). DPPH$^{\bullet}$ scavenging activity positively associates with neochlorogenic acid ($r=0.701$; $P\leq0.05$). Both neochlorogenic acid and DPPH$^{\bullet}$ scavenging activity were stable during storage, explaining their close association (Figure 1 a). The total polyphenolic content of the formulations was not significantly ($P>0.05$) associated with any of the other attributes.

The PCA scores plot (Figure 1 b) showed a separation between the formulations, with the 16 and 24% skin extract formulations being more closely associated with each other than with the 0% skin extract formulation. An increase in skin extract, a substitute for water in the 16 and 24% skin extract formulations, is responsible for this trend (Figure 1 b). No clear distinction is evident between treatments stored at 0 and 5°C for any of the formulations (Figure 1 b). PC1, furthermore, showed that the attributes, which were located exclusively in the right side of the plot (Figure 1 a), were mainly positively associated with the 16 and 24% skin extract formulations (Figure 1 b). These formulations thus possessed higher concentrations of polyphenolic compounds and higher antioxidant activity values than the 0% skin extract formulation. This was also observed in Chapter 3. Cheynier (2005) confirmed that the polyphenolic content of many fruits, such as red grapes, was higher in the skins than in the flesh. The formulations at a specific temperature follow a downward trend from 0 to 24 weeks storage time, from the top, right-hand part of the plot to the bottom, left-hand part of the plot (Figure 1 b). This indicated that polyphenolic compounds in the formulations decreased over 24 weeks. These results were especially pertinent to anthocyanins.

The anthocyanin content of plum juices decreased due to exposure to oxygen and ultraviolet light over time (Will & Dietrich, 2006). Polyphenolic compounds could remain stable during storage when the enzymes causing degradation are inactivated during pasteurisation (Will & Dietrich, 2006).

The heating of the plum nectar formulations during pasteurisation could also lead to the development of new compounds, such as anthocyanin condensation products, which may have higher antioxidant activities than the original polyphenolic compounds present in the fresh plums (Piga *et al*., 2003). This is likely to contribute to the stability of $DPPH^{\bullet}$ scavenging activity in nectar formulations despite a decreased anthocyanin content.

Phase 2

The purpose of Phase 2 of the shelf-life study was to determine the effect of storage on the sensory characteristics of the plum nectar formulations. The sensory results from Phase 2 of the shelf-life study (Figure 2) indicated no significant differences ($P>0.05$) in the plum flavour, plantlike flavour, raisin flavour, astringency, acidity, and sweetness over time for the 0 and 24% skin extract formulations. Differences observed between formulations for Phase 2 of the shelf-life study were similar to that observed in Chapter 3. In the current study, the 24% skin extract formulation had significantly ($P\leq0.05$) lower values for plum flavour, and higher values for plantlike flavour and astringency. Sweetness and acidity, however, were not significantly ($P>0.05$) different for formulations in Phase 2, unlike the higher acidity and lower sweetness levels observed for the 24% skin extract formulation compared to the 0% skin extract formulation in Chapter 3. These differences can be attributed to seasonal differences between plums obtained during the 2009 and 2010 harvest seasons.

The results from Phase 2 of the shelf-life study were unexpected, as the anthocyanin content of the nectar formulations, which were noted to decrease during storage, is linked to astringency (Brossaud *et al.,* 2001). Anthocyanins are known to interact strongly with other polyphenolic compounds in a sample, possibly altering the affinity of the polyphenolic compounds for salivary proteins (Brossaud *et al.,* 2001). The polymerisation and/or condensation of anthocyanins during storage are considered to decrease sensory astringency (Brossaud *et al*., 2001). In this study, the decrease in anthocyanin content was not sufficient to obtain a noticeable change in astringency as the shelf-life study progressed. Raisin flavour was thought to have developed during storage, as it was not noted in sensory analyses conducted in Chapter 3. The results from the ANOVA analysis conducted on the raisin flavour characteristic (Figure 2 c) indicated that this flavour was not related to storage time, as no significant ($P>0.05$) difference was found between the freshly prepared samples and samples stored up to 3 months at 5°C. The raisin flavour could be due to seasonal differences in the plums or differences in the grape concentrate used to prepare the formulations.

Chemical analyses (Figure 3) were also conducted on the formulations prepared in Phase 2 of the shelf-life study to validate the results from Phase 1 of the shelf-life study.

Results indicated that total polyphenolic content (Figure 3 b), quercetin-3-glucoside content (Figure 3 g), quercetin-3-xyloside content (Figure 3 h), quercetin-3-rutinoside content (Figure 3 i), and

the colour (Figure 3 j & k) underwent no significant ($P>0.05$) changes over time for both formulations. This suggests these attributes remained stable in the formulations during 3 months of storage. The total anthocyanin (Figure 3 a), neochlorogenic acid (Figure 3 d), cyanidin-3-glucoside (Figure 3 e), and cyanidin-3-rutinoside (Figure 3 f) contents underwent significant ($P\leq0.05$) changes for both formulations over time. These chemical attributes all decreased, with the exception of neochlorogenic acid (Figure 3 d), which increased during storage over time. The DPPH$^{\bullet}$ scavenging activity (Figure 3 c) only decreased over time for the 0% skin extract formulation. Significant ($P\leq0.05$) changes over time, however, were observed for total anthocyanin (Figure 4 a), neochlorogenic acid (Figure 4 d), cyanidin-3-glucoside (Figure 4 e), cyanidin-3-rutinoside (Figure 4 f) contents for the 0% skin extract formulation.

Of all the chemical attributes, DPPH$^{\bullet}$ scavenging activity (Figure 3 c) was the only attribute to display no significant ($P>0.05$) differences between the 0 and 24% skin extract formulations at the start of the shelf-life study. The 24% skin extract formulation had higher colour values and a higher polyphenolic content compared to the 0% skin extract formulation. These differences between formulations in Phase 2 of the shelf-life study were similar to both the results from Phase 1 of the shelf-life study (data not shown) and those observed in Chapter 3, except for the neochlorogenic acid content, which did not differ between the 0% and 24% skin extract formulations (Chapter 3). Most of the results for the chemical attributes from Phase 2 of the shelf-life study are similar to those of Phase 1, except for neochlorogenic acid, which was stable during storage in Phase 1, but increased during storage in Phase 2. The total and red colour values of formulations decreased during storage in Phase 1, while the no change was observed in Phase 2, possibly due to the shorter storage time.

CONCLUSION

Stability of the chemical attributes of the 0, 16, and 24% skin extract formulations were investigated during Phase 1 (24 weeks) of the shelf-life study. A decrease was observed in the red colour, as well as total and individual anthocyanin contents during 24 weeks of storage at 0 and 5°C. The content of the flavonols, quercetin-3-glucoside and quercetin-3-xyloside, also decreased during storage at 0°C, while total colour decreased during storage at 5°C. DPPH$^{\bullet}$ scavenging activity, total polyphenolic content, quercetin-3-rutinoside, and neochlorogenic acid were found to be stable over time for all formulation x temperature combinations.

The stability of the sensory characteristics (plum flavour, plantlike flavour, raisin flavour, sweetness, acidity, and astringency) of the 0 and 24% skin extract formulations were evaluated over 3 months of storage (Phase 2). Storage of the nectar formulations for 3 months did not affect their sensory attributes. Chemical analyses conducted on the formulations of Phase 2 of the shelf-life study, showed similar results to those obtained in Phase 1, validating these results.

The results from the shelf-life study are extremely positive for the juice industry, as the formulations compare well with the average red fruit commercial beverages (discussed in Chapter 3) and prove to have a stable shelf-life for at least 3 months, where flavour, taste and mouthfeel, as well as antioxidant activity are maintained. Very little change in colour was observed despite decreases in anthocyanin content.

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Table 1. List of chemicals and suppliers of the chemicals used in the chemical analyses of the commercial beverage and plum nectar samples

Chemical	Supplier
2,2'-Azo-bis-(2-methylpropionamidine) Dihydrochloride (AAPH), 97%	Aldrich; Steinheim, Germany
2,2-Diphenyl-1-picrylhydrazyl (DPPH)	Sigma, Sigma-Aldrich; Steinheim, Germany
6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox)	Aldrich; Steinheim, Germany
Acetonitrile, LiChrosolv gradient grade for liquid chromatography	Merck; Darmstadt, Germany
Anhydrous sodium carbonate	Saarchem, Merck; Gauteng, South Africa
Ascorbic acid	Sigma, Sigma-Aldrich; Steinheim, Germany
(+)-Catechin	Sigma, Sigma-Aldrich; Steinheim, Germany
Neochlorogenic acid	Phytolab; Vestenbergreath, Germany
Cyanidin-3-glucoside	Extrasynthese; Genay, France
Cyanidin-3-rutinoside	Extrasynthese; Genay, France
HCl, 37%	Saarchem Premium, Merck; Gauteng, South Africa
Folin-Ciocalteau reagent	Merck; Darmstadt, Germany
Formic acid, 98%	BDH; London, England
Methanol	Merck; Gauteng, South Africa
Quercetin-3-rutinoside	Sigma, Sigma-Aldrich; Steinheim, Germany
Quercetin-3-glucoside	Fluka Sigma-Aldrich; Steinheim, Germany

Table 2. The physicochemical characteristics of plum pulp and skin extract (2010 harvest)

	TSS	pH
PR05-17 pulp	12.23	3.1
PR05-17 skin extract	5.02	3.4

Total soluble solids (TSS) - °Brix

Table 3. Reference standards used in training phase of descriptive sensory analysis

Reference	Specifications
Pulp	20 mL Test batch pulp
Skin	20 mL Test batch skin extract
Grape concentrate	20 mL KWV grape concentrate diluted to 13 °Brix

Test batch - various yellow- & red-fleshed plums from Bienne Donné farm (Groot Drakenstein, South Africa); KWV - Koöperatieve Wijnbouwers Vereniging, South Africa.

Table 4. Regression coefficients (R^2) and corresponding probability values (P) of the different attributes for the plum nectar formulations during Phase 1 (24 weeks)

Formulation x temp	Colour		DPPH	Total Phenols	Total Anthos	HPLC					
	Total	Red (520 nm)				Cy-3-glc	Cy-3-rut	Q-3-rut	Q-3-glc	Q-3-xyl	NeoChA
0% skin extract (0℃)	0.006	0.232	0.194	0.019	0.703	0.928	0.928	0.081	0.792	0.794	0.369
	(P=0.69)	**(P<0.01)**	(P=0.20)	(P=0.70)	**(P<0.01)**	**(P<0.01)**	**(P<0.01)**	(P=0.43)	**(P<0.01)**	**(P<0.01)**	(P=0.06)
16% skin extract (0℃)	0.01	0.141	0.03	0.106	0.683	0.929	0.863	0.006	0.565	0.669	0.242
	(P=0.60)	**(P=0.04)**	(P=0.63)	(P=0.36)	**(P<0.01)**	**(P<0.01)**	**(P<0.01)**	(P=0.83)	**(P=0.01)**	**(P<0.01)**	(P=0.15)
24% skin extract (0℃)	0.294	0.573	0.343	0.048	0.95	0.9	0.885	0.025	0.629	0.703	0.385
	(P<0.01)	**(P<0.01)**	(P=0.08)	(P=0.55)	**(P<0.01)**	**(P<0.01)**	**(P<0.01)**	(P=0.66)	**(P<0.01)**	**(P<0.01)**	(P=0.06)
0% skin extract (5℃)	0.737	0.747	0.454	0.378	0.783	0.89	0.803	0.067	0.221	0.219	0.092
	(P<0.01)	**(P<0.01)**	**(P=0.03)**	(P=0.06)	**(P<0.01)**	**(P<0.01)**	**(P<0.01)**	(P=0.47)	(P=0.17)	(P=0.17)	(P=0.40)
16% skin extract (5℃)	0.471	0.627	0.069	0.301	0.805	0.859	0.843	0.29	0.191	0.134	0.042
	(P<0.01)	**(P<0.01)**	(P=0.46)	(P=0.10)	**(P<0.01)**	**(P<0.01)**	**(P<0.01)**	(P=0.11)	(P=0.21)	(P=0.30)	(P=0.57)
24% skin extract (5℃)	0.427	0.546	0.279	0.053	0.827	0.841	0.819	0.212	0.011	0.157	0.013
	(P<0.01)	**(P<0.01)**	(P=0.12)	(P=0.52)	**(P<0.01)**	**(P<0.01)**	**(P<0.01)**	(P=0.18)	(P=0.78)	(P=0.26)	(P=0.75)

Colour – Absorbance; DPPH - DPPH$^{\bullet}$ scavenging activity; Total Anthos - total anthocyanin content; Cy-3-glc – cyanidin-3-glucoside; Cy-3-rut – cyanidin-3-rutinoside; Q-3-glc – quercetin-3-glucoside; Q-3-xyl – quercetin-3-xyloside; NeoChA - neochlorogenic acid.

Table 5. ANOVA on the regression slopes to compare the nectar formulations during Phase 1 (24 weeks)

Formulation x temp	Colour		Total Anthos	HPLC			
	Total	Red (520 nm)		Cy-3-glc	Cy-3-rut	Q-3-glc	Q-3-xyl
0% skin extract (0℃)	-0.0114^{a}	-0.0027^{a}	-0.0961^{a}	-0.1796^{ab}	-0.2667^{a}	-0.0196^{b}	-0.0183^{a}
16% skin extract (0℃)	0.0169^{a}	-0.0021^{a}	-0.1221^{ab}	-0.1990^{abc}	-0.3016^{ab}	-0.0262^{bc}	-0.0422^{b}
24% skin extract (0℃)	-0.0882^{b}	-0.0055^{b}	-0.1431^{ab}	-0.2157^{cd}	-0.3021^{ab}	-0.0305^{c}	-0.0488^{b}
0% skin extract (5℃)	-0.2109^{d}	-0.0083^{c}	-0.2097^{b}	-0.1728^{a}	-0.2562^{a}	-0.0075^{a}	-0.0060^{a}
16% skin extract (5℃)	-0.1660^{c}	-0.0074^{c}	-0.1768^{ab}	-0.2434^{d}	-0.3488^{b}	-0.0275^{bc}	-0.0176^{a}
24% skin extract (5℃)	-0.2290^{d}	-0.0089^{c}	-0.1809^{ab}	-0.2124^{bcd}	-0.2947^{ab}	0.0008^{a}	-0.0138^{a}
LSD (P = 0.05)	0.044	0.002	0.102	0.034	0.061	0.011	0.016
P-values	0.1511	0.0749	0.506	0.0655	0.2923	0.0018	0.1393

LSD – Least significant difference at the 5% level of significance. Colour – Absorbance; Total Anthos - Total anthocyanin content; Cy-3-glc – cyanidin-3-glucoside; Cy-3-rut – cyanidin-3-rutinoside; Q-3-glc – quercetin-3-glucoside; Q-3-xyl – quercetin-3-xyloside.

Table 6. The percentage change in the chemical attributes of the formulations stored at 0 and 5℃ during Phase 1 compared to the start of the shelf-life study

		Colour					HPLC					
Formulations x temp	**Time (Weeks)**	**Total**	**Red (520 nm)**	**DPPH**	**Total Phenols**	**Total Anthos**	**Cy-3-glc**	**Cy-3-rut**	**Q-3-rut**	**Q-3-glc**	**Q-3-xyl**	**NeoChA**
0% skin extract (0℃)	12	-2.17	-5.83	-12.66	-32.06	-0.18	-29.80	-25.82	-0.72	-0.74	-10.53	-18.31
	24	-8.66	-7.80	-20.98	-63.08	-15.75	-53.49	-48.72	-12.33	-15.55	-21.86	-23.56
16% skin extract (0℃)	12	-0.90	-0.51	-9.96	15.20	-9.07	-28.77	-29.44	-4.08	-9.27	-18.27	-13.36
	24	-11.95	-4.21	-9.32	-44.66	-14.02	-45.11	-43.83	-4.97	-10.00	-16.82	-15.69
24% skin extract (0℃)	12	-5.71	-13.56	-15.70	-29.86	-8.74	-30.15	-25.69	-4.89	-9.80	-20.91	-13.89
	24	-6.05	-16.41	-19.67	-26.91	-18.98	-44.49	-39.86	-8.04	-11.76	-18.75	-19.73
0% skin extract (5℃)	12	-22.95	-27.45	-29.36	-22.64	-22.64	-39.07	-34.69	-3.04	-5.49	-15.15	-15.07
	24	-32.77	-33.33	-24.67	-24.48	-24.48	-49.65	-36.27	-0.61	-1.57	-3.79	-11.86
16% skin extract (5℃)	12	4.68	-5.88	-16.86	-21.85	-21.85	-37.29	-32.68	-7.02	-8.55	-14.94	-19.53
	24	-17.76	-25.49	-18.57	-34.79	-34.79	-43.79	-38.57	-21.55	-7.94	-7.82	-10.22
24% skin extract (5℃)	12	-2.51	-9.80	-5.61	-16.35	-16.35	-33.68	-28.95	-6.41	-12.00	-25.22	-18.30
	24	-15.84	-21.57	6.14	-17.43	-17.43	-40.19	-34.59	1.47	0.83	-6.13	-9.06

Colour – Absorbance; DPPH - DPPH$^{\bullet}$ scavenging activity in µmol Trolox equivalents/L; Total Phenols - mg gallic acid equivalents/L; Total Anthos - Total anthocyanin content; Cy-3-glc – cyanidin-3-glucoside; Cy-3-rut – cyanidin-3-rutinoside; Q-3-rut – quercetin-3-rutinoside; Q-3-glc – quercetin-3-glucoside; Q-3-xyl – quercetin-3-xyloside; NeoChA – neochlorogenic acid.

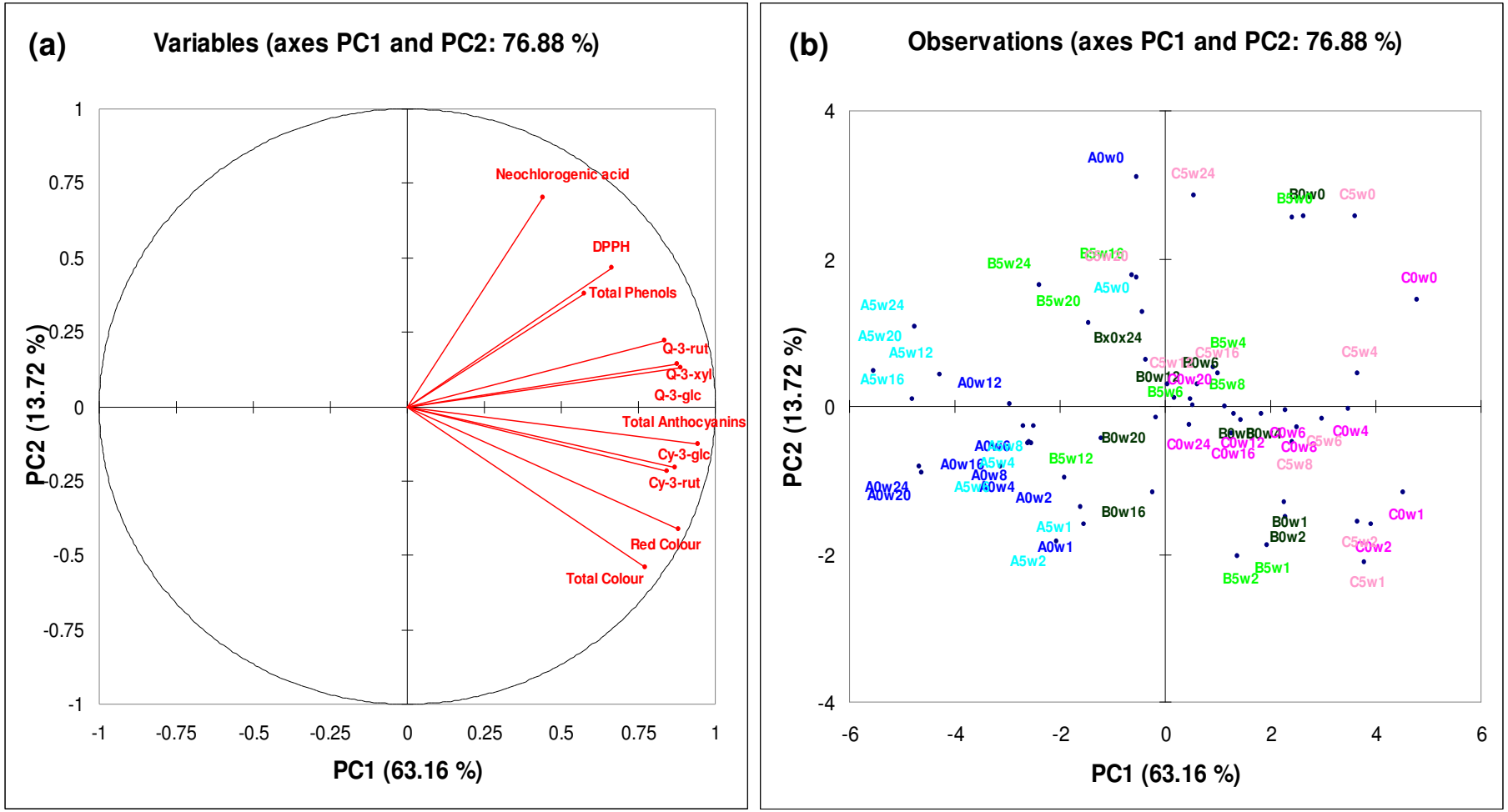


Figure 1 (a) PCA loadings plot of the chemical analyses attributes of the plum nectar formulations (0, 16 and 24% skin extract formulations) stored for 24 weeks at 0℃ and 5℃. **(b)** The PCA scores plot of the 0, 16 and 24% skin extract formulations. PC1 and PC2 explain 77.11% of the variance. Treatments are labelled e.g. A5wk20, where A represents the formulation, 5 the storage temperature, and wk20 the time point. The A, B and C represent the 0, 16, and 24% skin extract formulations, respectively. The blue, green and pink clusters of treatments are the 0, 16, and 24% skin extract formulations, respectively. The light and dark colours indicate formulations stored at 5℃ and 0℃.

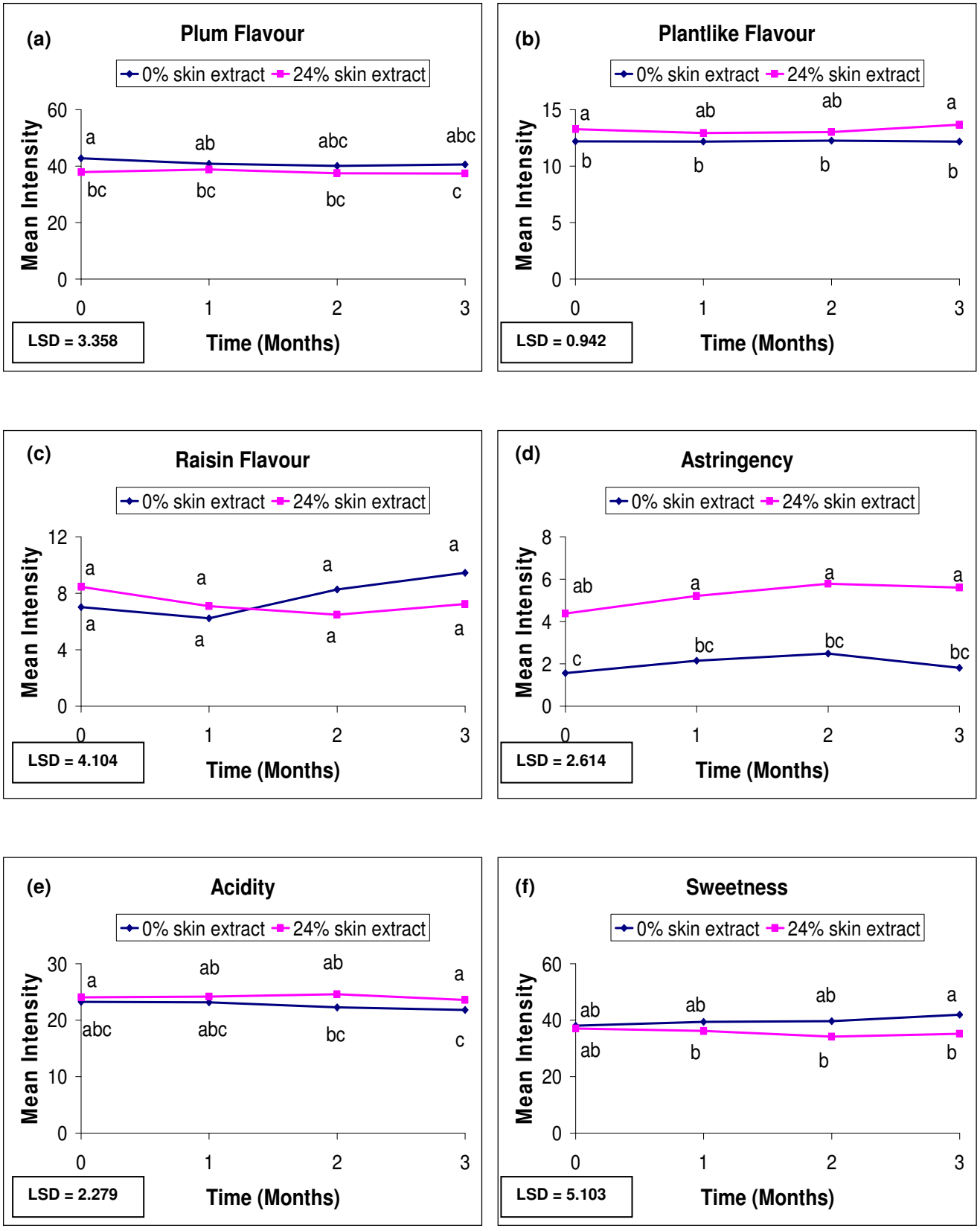
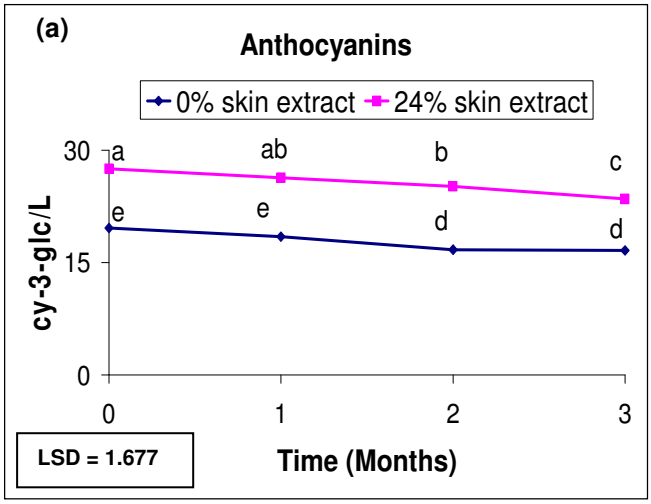
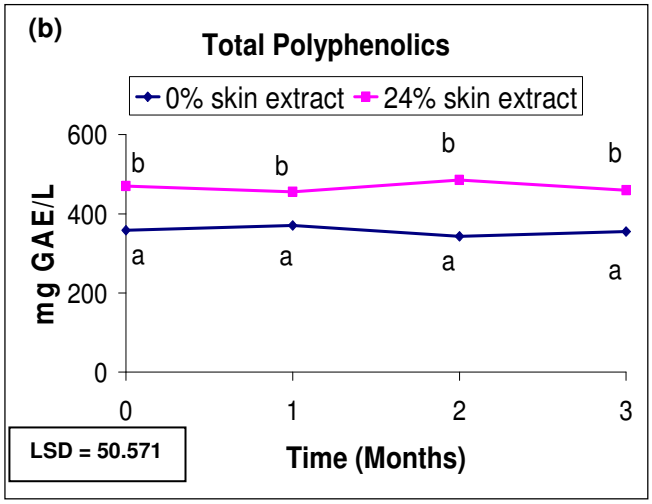
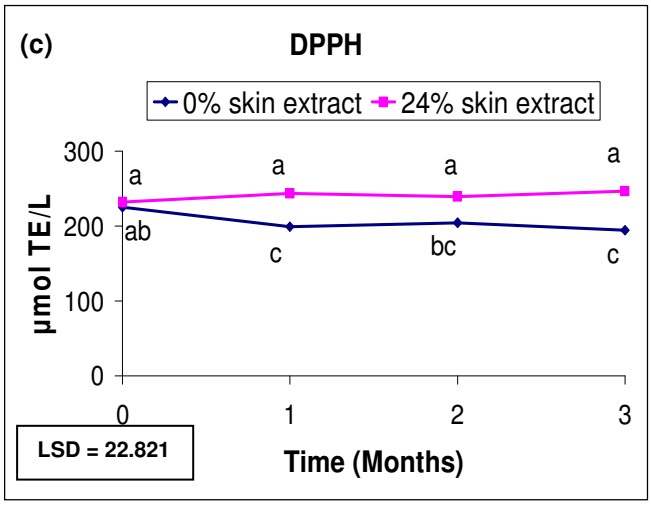
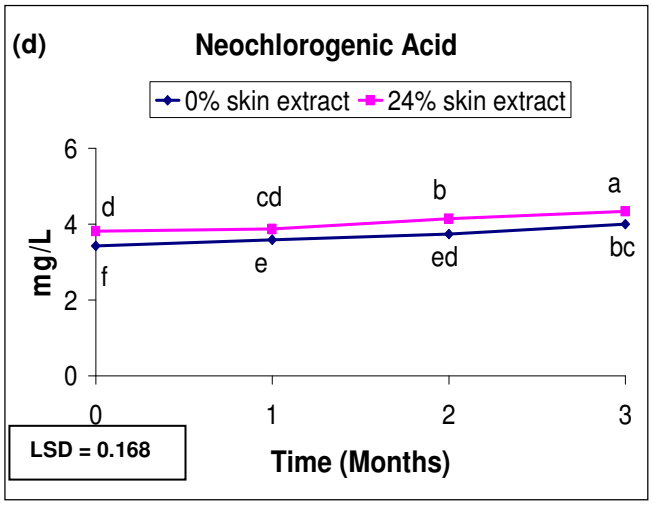


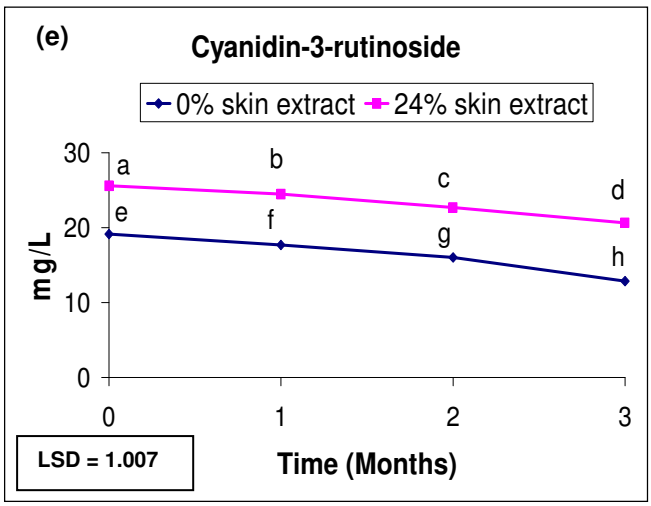
Figure 2 (a) Plum flavour, **(b)** plantlike flavour, **(c)** raisin flavour, **(d)** astringency, **(e)** acidity, and **(f)** sweetness of the 0 and 24% skin extract formulations of Phase 2 of the shelf-life study (3 months). LSD – Least significant difference at P = 0.05.

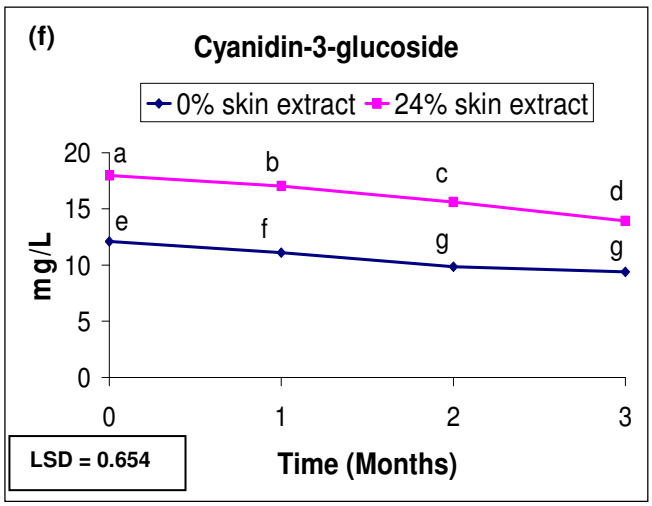

(a)
Anthocyanins
0% skin extract
24% skin extract
cy-3-glc/L
30
15
0
a
ab
b
c
e
e
d
d
0
1
2
3
LSD = 1.677
Time (Months)

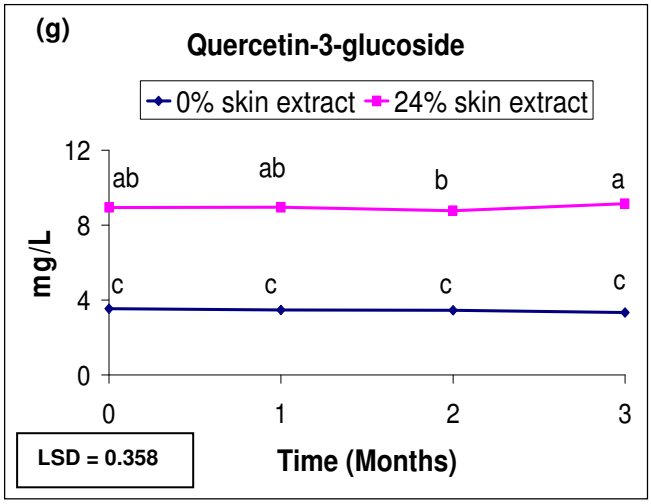

(b)
Total Polyphenolics
0% skin extract
24% skin extract
mg GAE/L
600
400
200
0
b
b
b
b
a
a
a
a
0
1
2
3
LSD = 50.571
Time (Months)

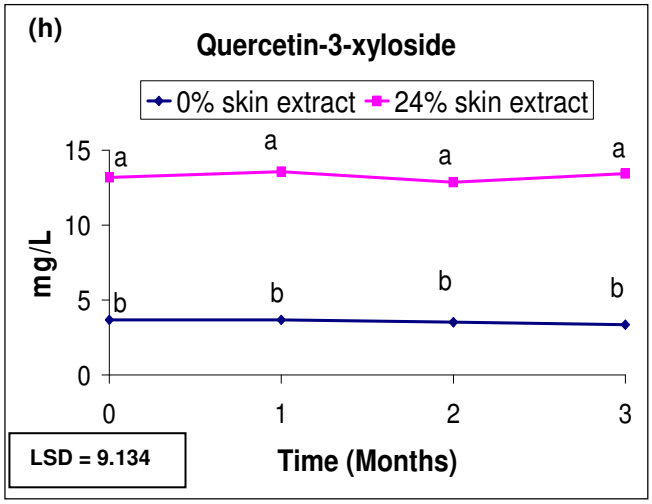

(c)
DPPH
0% skin extract
24% skin extract
µmol TE/L
300
200
100
0
a
a
a
a
ab
c
bc
c
0
1
2
3
LSD = 22.821
Time (Months)

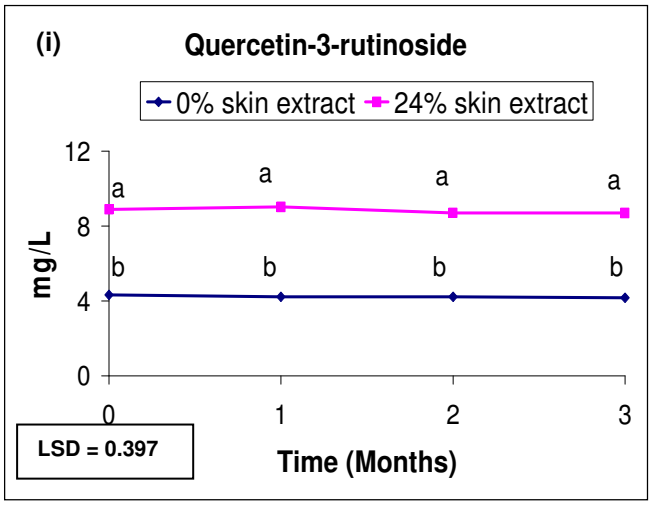

(d)
Neochlorogenic Acid
0% skin extract
24% skin extract
mg/L
6
4
2
0
d
cd
b
a
f
e
ed
bc
0
1
2
3
LSD = 0.168
Time (Months)

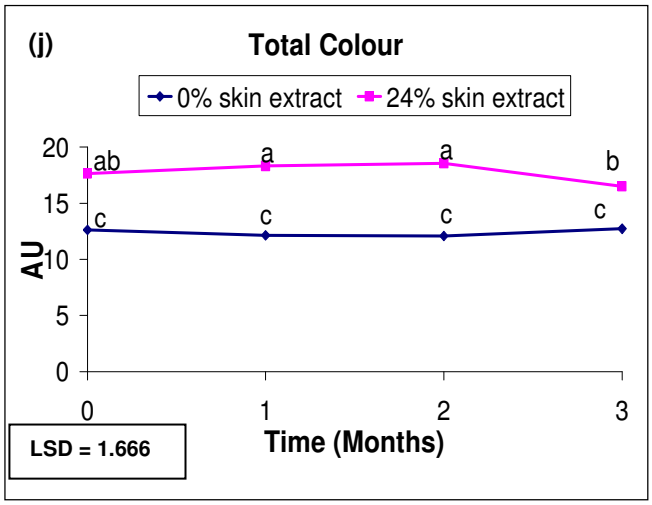

(e)
Cyanidin-3-rutinoside
0% skin extract
24% skin extract
mg/L
30
20
10
0
a
b
c
d
e
f
g
h
0
1
2
3
LSD = 1.007
Time (Months)


(f)
Cyanidin-3-glucoside
0% skin extract
24% skin extract
mg/L
20
15
10
5
0
a
b
c
d
e
f
g
g
0
1
2
3
LSD = 0.654
Time (Months)









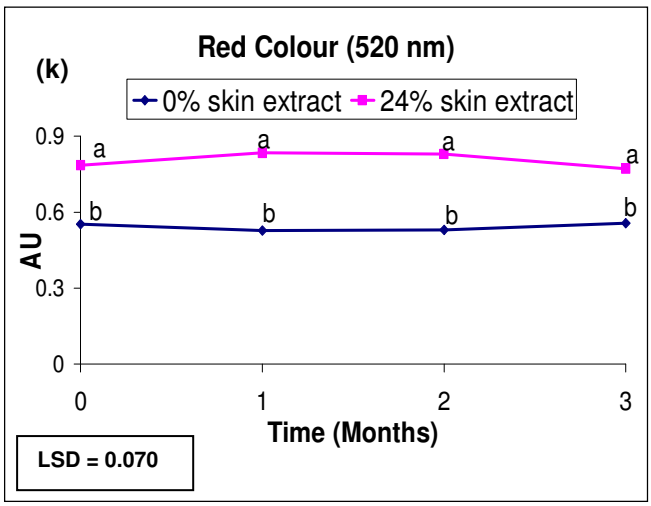


Figure 3 **(a)** Spectrophotometric anthocyanin analysis, **(b)** total polyphenolic content, **(c)** DPPH radical scavenging activity, and **(d)** quantitative neochlorogenic acid, **(e)** cyanidin-3-glucoside, **(f)** cyanidin-3-rutinoside, **(g)** quercetin-3-glucoside, **(h)** quercetin-3-xyloside, **(i)** quercetin-3-rutinoside **(j)** total colour, and **(k)** red colour at 520 nm of the 0 and 24% skin extract formulations of Phase 2 of the shelf-life study (3 months). LSD – Least significant difference at P = 0.05.

Chapter 5
General discussion and conclusions

With a global consciousness of health increasing, polyphenolic compounds are becoming evermore important in product development. The potential health benefits of polyphenolic compounds are being recognised more progressively, as reports indicate that these compounds inhibit the harmful effects of reactive oxygen species, which act as oxidants (Aquilano *et al*., 2008). Polyphenolic compounds are associated with a decreased risk of chronic or lifestyle diseases, such as atherosclerosis (Kehrer & Smith, 1994), cancer (Thomasset *et al*., 2006), heart disease (Chong *et al*., 2010), and degenerative diseases (Aquilano *et al*., 2008). These compounds are considered to possibly have antiulcer, antispasmodic, antisecretory, antidiarrhoeal (Carlo *et al*., 1999), and antihepatotoxic properties (Hemingway & Larks, 1988). The health promoting properties of polyphenolic compounds have prompted fruit breeders and food manufacturers to research and develop food products that are high in polyphenolic compounds. Fruits and fruit beverages have great potential in this regard.

The global fruit beverage market is a large, ever-growing market, which generated a total revenue of $69,357.4 billion in 2009 (Anon., 2010). Fruit drinks were found to constitute the largest segment of the global fruit beverage market (27.8% of total market value), with nectars only making up 16.3% of total market value (Anon., 2010). This indicates a major potential in the market for the production of fruit nectars, both globally and in South Africa. Globally, an increase in the production of fruit beverages containing polyphenolic compounds, such as those produced from cranberries, pomegranates and strawberries, has also been observed (Anon., 2010).

The opportunity to produce a fruit beverage with a high polyphenolic content was prompted by, not only the phenomenal increase in traditional fruit beverage production in South Africa (Deciduous Fruit Producers' Trust, 2009), but also the demand for fruit beverages with a substantial polyphenolic content (Kalt, 2005). Fruit beverages produced from fresh red-fleshed plums are currently not readily available in South Africa. This was interesting, as red-fleshed plums are considered to have a high polyphenolic content (Cevallos-Casals *et al*., 2006). Total plum production in South Africa has undergone a steady increase from 32.9 tons in 2000 to 62.6 tons in 2008. However, only a small percentage of these plums undergo processing (Deciduous Fruit Producers' Trust, 2009).

In South Africa, seven different classes for fruit beverages are currently being regulated by the Department of Agriculture and Fisheries (1980). These include fresh fruit juice, unsweetened juice, sweetened juice, nectar, squash, and drink. Fruit nectars are allowed to contain less than 100% fruit juice, with the amount determined by the fruit type. No specific regulations are mentioned for plum beverages. Beverages from plums are thus classified as 'from unspecified fruits' (Department of Agriculture and Fisheries, 1980). Plum skins, which would normally go to

waste during processing, contain polyphenolic compounds in large concentrations (Donovan *et al*., 1998). An aqueous extract could be developed to recover some of these polyphenolic compounds that would otherwise be lost. To produce nectar with a high polyphenolic content and potential health benefits, an aqueous extract from the plum skins can easily be added to the plum pulp.

The aims of the research project were to develop a red-fleshed functional plum beverage with a high total polyphenolic content, characterisation of the beverage in terms of sensory profile, consumer acceptance, colour, polyphenolic content, and antioxidant activity and benchmarking of the beverage against similar commercial beverages (Chapter 3). A further aim was to evaluate the shelf-life stability of the red-fleshed plum beverage in terms of sensory profile, colour, polyphenolic content and antioxidant capacity (Chapter 4).

During product development of the plum nectar formulations, different juice concentrates were tested using varying amounts of pulp, apple concentrate and deionised water. The beverage formulations were evaluated by an in-house expert panel and the optimum combination was determined as 40% plum pulp with varying amounts of skin extract adjusted to 13 °Brix using grape concentrate (sourced from KWV, Paarl). Obtaining a suitable concentrate, to correct for the total soluble solids of the formulations, was challenging, as a concentrate with a high total soluble solids and low titratable acidity content were needed. The concentrate chosen was deemed to have a low impact on the nectar flavour by an in-house expert panel. Multiple methods for skin extract preparation were tried. In the optimum method, the skins were blended with deionised water (1:2) for 1 min before being subjected to vacuum-pump filtration.

Sensory descriptive analysis and consumer analysis was conducted on the nectar formulations, containing 0, 8, 16, 24, and 32% skin extract, to determine the effect of the skin extract content on sensory descriptors and consumer preference (Chapter 3). It was found that the samples containing the least amount of skin extract were more preferred than those containing high levels of skin extract. High skin extract contents (32%) caused a decrease in consumer preference, as this increased the intensity of negative sensory descriptors, namely plantlike aroma, plantlike flavour, acidity and astringency. With an increase in skin extract, a decrease was also noted in intensity of the positive sensory descriptors, namely plum aroma, plum flavour, and sweetness. The 32% skin extract formulation was least preferred by consumers. All of the formulations were, however, considered acceptable, as 83% of consumers rated the nectar formulations between *Like slightly* and *Like extremely*. The 0, 8, 16 and 24% skin extract formulations received high ratings between *Like slightly* and *Like extremely*, while the 32% skin extract formulation was given the lowest rating between *Like slightly* and *Like extremely* and the highest score for *Dislike slightly.*

Chemical (colour, antioxidant activity, total polyphenolic content, total anthocyanin content, and individual polyphenolic compound content) analyses conducted on the plum nectar formulations indicated an increase in colour, antioxidant activity, and polyphenolic content with an increase in skin extract content (Chapter 3). Similar chemical analyses were conducted on a

selection of commercial beverages containing so-called “superfruits”, namely pomegranate, strawberry, blueberry, and cranberry. Commercial beverage results indicated that the plum nectar formulations were similar or superior in colour, antioxidant activity, and polyphenolic content.

A shelf-life study was also conducted on the plum nectar formulations (Chapter 4). This was conducted in two phases: in Phase 1 the effect of a 24-week storage period on the colour, antioxidant activity, total polyphenolic content, total anthocyanin content, and individual polyphenolic compound content was investigated. In Phase 2 the effect of a 3-month storage period on the sensory attributes was tested.

The total and individual anthocyanins, as well as the red colour of the plum nectars significantly ($P \leq 0.05$) decreased during storage in Phase 1 at both temperatures, while the other chemical attributes remained stable. Quercetin-3-glucoside and quercetin-3-xyloside were deemed unstable during storage at 0°C. Similar results were found for the total colour of the formulations stored at 5°C.

During Phase 2 of the shelf-life study, it was noted that the individual anthocyanins, as well as the total anthocyanins decreased during 3 months at 5°C of storage, while neochlorogenic acid increased. The total polyphenolic content, flavonol content, and red colour of the formulations were stable over time for both formulations. Results from the study indicated that the sensory attributes of the nectar formulations were similar to those determined in Chapter 3. The sensory attributes did not undergo significant ($P > 0.05$) changes during three months of storage. There were also no developments of any new flavours that could be linked to storage. The raisin flavour, which was found in the formulations in Chapter 4, but not Chapter 3, is considered to have occurred due to seasonal changes in the fruit or variations in the grape concentrate used in the preparation of the nectar formulations.

The results from the shelf-life study are extremely positive for the juice industry, as the formulations compare well with the average red fruit commercial beverages (discussed in Chapter 3) and prove to have a stable shelf-life for at least 3 months, where flavour, taste and mouthfeel, as well as antioxidant activity are maintained (Chapter 3). Very little change in colour was observed despite decreases in anthocyanin content. Chemical results from Phase 2 verified those of Phase 1.

The challenges regarding the study included the limited amount of plums available for the study within each harvest season. This problem was overcome through careful management of the available fruit and the use of other plum cultivars during the development of the plum nectar formulations and the skin extraction method. Variations in the results for some of the analyses were found between the 2009 and 2010 harvests. These were attributed to seasonal influences of the fruit and changes in the grape concentrate used in the preparation of the nectar formulations. The negative sensory attributes that are associated with the plum skin extract and the polyphenolic compounds contributed by the extract were considered challenging, as the correct balance between total soluble solids, skin extract and pulp content had to be determined. The high titratable

acidity of the plums was also problematic. This was overcome by formulating the nectar with a low acid, high total soluble solids grape concentrate. Differences in chemical analysis methods for similar aspects, such as total anthocyanin determination vs. individual anthocyanin determination yielded differing results. Determining which results were most accurate, proved challenging. The results from the total anthocyanin assay were lower than the combination of the individual anthocyanins. The antioxidant activity assays, the ORAC, DPPH$^{\bullet}$ scavenging activity, and FRAP assays, all gave varying results. The ORAC gave the largest values, followed by FRAP, and DPPH$^{\bullet}$ scavenging activity, respectively, in µmol Trolox equivalents/L. These variations in results were consistent throughout the study and were considered as separate attributes to avoid confusion.

Future research can be conducted on a broader spectrum of high antioxidant, commercially available beverages to get a better idea of where the plum nectar formulations should be benchmarked. Stellenbosch possibly has a limited range of fruit beverages commercially available compared to the rest of South Africa or the world. Beverages from fruits, such as cherries, were not examined for their antioxidant activity or polyphenolic contents, but are commercially available in South Africa, while beverages prepared from fruits, such as mangosteen, have been found in countries, such as Malaysia and Sri Lanka (Pedraza-Chaverri *et al*., 2008). These fruits are considered to have high antioxidant activities and polyphenolic contents (Pedraza-Chaverri *et al*., 2008). Thus, including them in the benchmarking of the plum nectar formulations could yield different results, compared to these found in this study. Methods on how to improve consumer acceptance, in spite of the negative sensory attributes brought on by polyphenolic compounds, can be explored to produce a beverage with a much higher polyphenolic content, which is deemed beneficial to consumer health. This could be done by adding compounds to the plum nectar formulations, such as carboxymethyl cellulose, which masks the astringency of polyphenolic compounds (Troszyńska *et al*., 2008).

Other processing techniques, such as those involving enzymes and clarification agents (Chang *et al*., 1994) can be tested to determine whether they will have a greater total polyphenolic content yield. Chang *et al*. (1994) prepared a juice by crushing plums and adding Clarex® L (Solvay Enzymes Inc., Elkhart, USA), which aided juice extraction. Thereafter, the macerate was heated before undergoing pressing. Sodium bentonite and gelatine solutions were then added to the juice to facilitate clarification. The mixture was left overnight before racking, filtration, and pasteurisation. To improve the stability of the anthocyanins in the plum nectar formulations, a pigment-improving agent can be added to the formulation (Lenoble *et al*., 1999). A pigment-improving agent, such as the one patented by Lenobel *et al*. (1999), improves anthocyanin stability in the presence of light, heat, and/or pH increases (Lenoble *et al*., 1999).

Additional future research could be conducted on the other, less prominent, individual polyphenolic compounds in the nectar formulations, such as 3-*O*-caffeoylshikimic acid, 3-*O*-feruloylquinic acid, kaempferol-3-rutinoside, kaempferol-3-glucoside, kaempferol-3-galactoside,

kaempferol-3-arabinoside-7-rhamnoside (Nunes *et al*., 2008), (+)-catechin gallate, (+)-gallocatechin gallate, and (+)-gallocatechin (Medina *et al*., 2007) using HPLC. The effect of these compounds on the total polyphenolic content can thus be taken into account.

The relationships between the polyphenolic compounds in the plum nectar formulations can also be explored in greater detail. In this study, an association between the total anthocyanins and the individual anthocyanins were noticed. The extent of their interaction was, however, not analysed. González-Manzano *et al.* (2008) found that anthocyanin copigmentation with flavan-3-ols occurred slowly and thermodynamically, rather than kinetically. The greatest copigments were obtained from oligomers, while the poorest were obtained from monomers (González-Manzano *et al.,* 2008).

Plums are known to not only contain various sugars, acids, pectins, tannins and enzymes, but also polyphenolic compounds (Walkowiak-Tomczak *et al*., 2008). Future research could also be conducted on the specific sugars, protein, and minerals contents of the nectar formulations. The analyses for these attributes include HPLC or gas chromatography (Morel du Boil & Schäffler, 1991), the Kjeldahl method (Ligugnana, 1976), and capillary electrophoresis (Yang *et al*., 1995). The analyses could also be performed on the separate ingredients of the nectars, namely the plum pulp, the plum skin extract and the grape concentrate.

The influences of different forms of packaging could also be analysed with respect to the degradation of polyphenolic compounds. Pérez-Vicente *et al*. (2004) found that the anthocyanins of pomegranate juice degraded in glass packaging due to exposure to light, while experiencing greater degradation in paperboard carton packaging due to the oxygen introduced through the permeable material. The type of packaging can thus influence the total polyphenolic content of the plum nectar formulation during storage.

Lastly, the influences of the supply chain and the way these influence affect polyphenolic degradation could also be analysed, as processing conditions, such as slicing, peeling, high temperatures, oxygen, and light could adversely affect the polyphenolic content of the plum nectar formulations (Piga *et al*., 2003).

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ADDENDUM 1

JUDGE NO:	NAME OF PANEL MEMBER:	Replication
PAGE 1	0|____10_____20____30_____40_____50_____60____70____80____90____|100	

PLUM AROMA

None 0 _ 100 Prominent

0% 0M 0|_____|__100

0% 1M 0|_____|__100

0% 2M 0|_____|__100

0% 3M 0|_____|__100

24% 0M 0|_____|__100

24% 1M 0|_____|__100

24% 2M 0|_____|__100

24% 3M 0|_____|__100

PLANTLIKE AROMA

None 0 __ 100 Prominent

0% 0M 0|_____|__100

0% 1M 0|_____|__100

0% 2M 0|_____|__100

0% 3M 0|_____|__100

24% 0M 0|_____|__100

24% 1M 0|_____|__100

24% 2M 0|_____|__100

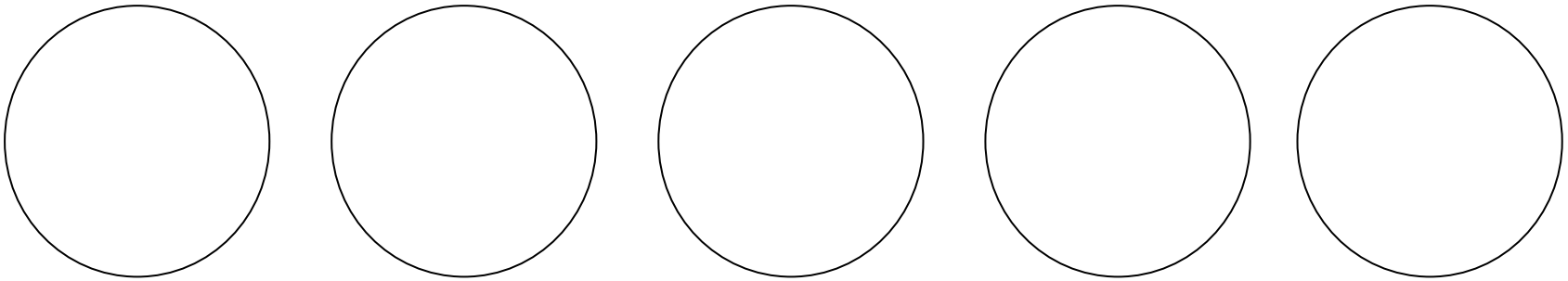
24% 3M 0|_____|__100

PAGE 2	0|____10_____20____30_____40_____50_____60____70____80____90____|100
PLUM FLAVOUR **None 0 _ 100 Prominent**	**0% 0M** 0|_____|__100 **0% 1M** 0|_____|__100 **0% 2M** 0|_____|__100 **0% 3M** 0|_____|__100 **24% 0M** 0|_____|__100 **24% 1M** 0|_____|__100 **24% 2M** 0|_____|__100 **24% 3M** 0|_____|__100
PLANTLIKE FLAVOUR **None 0 __ 100 Prominent**	**0% 0M** 0|_____|__100 **0% 1M** 0|_____|__100 **0% 2M** 0|_____|__100 **0% 3M** 0|_____|__100 **24% 0M** 0|_____|__100 **24% 1M** 0|_____|__100 **24% 2M** 0|_____|__100 **24% 3M** 0|_____|__100

PAGE 3	0|____10_____20____30_____40_____50_____60____70____80____90____|100
SWEETNESS **None 0 _ 100 Prominent**	**0% 0M** 0|_____|__100 **0% 1M** 0|_____|__100 **0% 2M** 0|_____|__100 **0% 3M** 0|_____|__100 **24% 0M** 0|_____|__100 **24% 1M** 0|_____|__100 **24% 2M** 0|_____|__100 **24% 3M** 0|_____|__100
ACIDITY **None 0 __ 100 Prominent**	**0% 0M** 0|_____|__100 **0% 1M** 0|_____|__100 **0% 2M** 0|_____|__100 **0% 3M** 0|_____|__100 **24% 0M** 0|_____|__100 **24% 1M** 0|_____|__100 **24% 2M** 0|_____|__100 **24% 3M** 0|_____|__100

PAGE 4	0|____10_____20____30_____40_____50_____60____70____80____90____|100
ASTRINGENCY **None 0 _ 100 Prominent**	**0% 0M** 0|_____|__100 **0% 1M** 0|_____|__100 **0% 2M** 0|_____|__100 **0% 3M** 0|_____|__100 **24% 0M** 0|_____|__100 **24% 1M** 0|_____|__100 **24% 2M** 0|_____|__100 **24% 3M** 0|_____|__100

ADDENDUM 2



ACCEPTABILITY OF FIVE PLUM JUICE SAMPLES

NAME OF JUDGE: _____ **JUDGE NO:**_____

PLEASE **CIRCLE** WHICHEVER IS APPLICABLE:

GENDER:	*AGE:*	*CONSUMPTION OF FRUIT JUICE:*
• ***Male***	• ***18-25***	• ***7x per week***
• ***Female***	• ***26-30***	• ***3x per week***
	• ***31-40***	• ***1x per week***
	• ***41-45***	• ***2x per month***
	• ***46+***	• ***Less than 1x per month***
		• ***NEVER***

INSTRUCTIONS

- PLEASE TASTE THE 5 SAMPLES FROM **LEFT TO RIGHT**.
- **RINSE YOUR MOUTH WITH** WATER **BEFORE BEGINNING. RINSE YOUR MOUTH BETWEEN THE SAMPLES. TAKE A** GENEROUS SIP **FROM EACH SAMPLE**
- **RANK THE SAMPLES ON THE FOLLOWING SCALE. IN EACH CASE,** CIRCLE THE NUMBER NEXT TO THE PREFERRED DEGREE OF LIKING

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

THANK YOU VERY MUCH FOR YOUR INVALUABLE ASSISTANCE, PLEASE COLLECT A SMALL "GIFT" AS YOU LEAVE THE SENSORY AREA

ADDENDUM 3

JUDGE NO:	NAME OF PANEL MEMBER:	Replication
PAGE 1	0|____10_____20____30_____40_____50_____60____70____80____90____|100	

PLUM FLAVOUR

None 0 _ 100 Prominent

0% 0M 0|_____|__100

0% 1M 0|_____|__100

0% 2M 0|_____|__100

0% 3M 0|_____|__100

24% 0M 0|_____|__100

24% 1M 0|_____|__100

24% 2M 0|_____|__100

24% 3M 0|_____|__100

PLANTLIKE FLAVOUR

None 0 __ 100 Prominent

0% 0M 0|_____|__100

0% 1M 0|_____|__100

0% 2M 0|_____|__100

0% 3M 0|_____|__100

24% 0M 0|_____|__100

24% 1M 0|_____|__100

24% 2M 0|_____|__100

24% 3M 0|_____|__100

PAGE 2	0|____10_____20____30_____40_____50_____60____70____80____90____|100
RAISIN FLAVOUR **None 0 _ 100 Prominent**	**0% 0M** 0|_____|__100 **0% 1M** 0|_____|__100 **0% 2M** 0|_____|__100 **0% 3M** 0|_____|__100 **24% 0M** 0|_____|__100 **24% 1M** 0|_____|__100 **24% 2M** 0|_____|__100 **24% 3M** 0|_____|__100
SWEETNESS **None 0 __ 100 Prominent**	**0% 0M** 0|_____|__100 **0% 1M** 0|_____|__100 **0% 2M** 0|_____|__100 **0% 3M** 0|_____|__100 **24% 0M** 0|_____|__100 **24% 1M** 0|_____|__100 **24% 2M** 0|_____|__100 **24% 3M** 0|_____|__100

PAGE 3	0|____10_____20____30_____40_____50_____60____70____80____90____|100
ACIDITY **None 0 _ 100 Prominent**	**0% 0M** 0|_____|__100 **0% 1M** 0|_____|__100 **0% 2M** 0|_____|__100 **0% 3M** 0|_____|__100 **24% 0M** 0|_____|__100 **24% 1M** 0|_____|__100 **24% 2M** 0|_____|__100 **24% 3M** 0|_____|__100
ASTRINGENCY **None 0 __ 100 Prominent**	**0% 0M** 0|_____|__100 **0% 1M** 0|_____|__100 **0% 2M** 0|_____|__100 **0% 3M** 0|_____|__100 **24% 0M** 0|_____|__100 **24% 1M** 0|_____|__100 **24% 2M** 0|_____|__100 **24% 3M** 0|_____|__100