# Application of innovative beverage fermentation technology to plums and selected berries

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

**Master of Technology (Food Technology)**

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**September 2016**

# DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that it has not previously, in its entire or part, been submitted at any other university for a degree.

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# ABSTRACT

This study focused on alcoholic fermented fruit beverages that were produced from various types of fruit, value addition and thus potentially increasing the diversity of commercially available fruit wines. Non-grape alcoholic fermented fruit beverages is a complex mixture of water, alcohol, and other components, that are either initially present in the fruit, or are formed during the fermentation process. The evaluation of wine and similar fermented products quality is important for manufacturers and consumers. The routine analysis of alcoholic fermented fruit beverages acts as an important tool that is useful for wine classification, quality control and sensory evaluation. Therefore, the aims of this study were (1) to measure methanol, ethanol, titratable acidity, objective colour, total soluble solids and sensory profile as a function of yeast strain and percentage pulp in order to adapt existing technologies toward producing new fermented fruit beverage products using plums, an under-utilized agricultural produce; and (2) to measure methanol, ethanol, titratable acidity, objective colour, total soluble solids and sensory profile as a function of yeast strain, pulp percentage and sugar levels in order to adapt existing technologies toward producing new fermented fruit beverages based on red and white wine styles, while applying the technology developed in the first part of the study using red-fleshed plums, blueberries and blackberries. The independent variables (ID) were yeast strains (1) *Saccharomyces cerevisiae* VIN13, (2) *Saccharomyces cerevisiae* NT116, and (3) *Saccharomyces bayanus* N96, with formulations containing percentage pulp concentrations at (40%, 50% and 60%). The dependent variables (DV) constituted key quality parameters for white and red wine style, namely methanol, ethanol, titratable acidity, objective colour, total soluble solids, pH and sensory profile were measured. The optimal combination of independent variables was ascertained and in terms of the overall consumer response, for the red-fleshed plum beverage sample treatment N 96, 60% pulp showed the highest preference amongst consumers. In terms of the other dependent variables, namely methanol, ethanol, titratable acidity, objective colour, total soluble solids, pH and sensory profiles of alcoholic fermented fruit beverages based on white and red wine styles. The processing conditions developed and applied in this study towards the development of alcoholic fermented beverages utilizing plums and selected berries demonstrated ways of improving the utilization of fruit commodities by developing niche products. Hence, the development of alcoholic fermented beverages utilizing (plums and selected berries) showed potential for micro agro-industries, as well as the impact on its potential role in employment creation and income generation.

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Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

# ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the following people and institutions that formed an integral part of the completion of this research study:

Prof. J. Van Wyk, my supervisor and Head of Department at the Cape Peninsula University of Technology, for her guidance, patience, support and assistance during the course of my studies;

Dr C.F. Hansmann, my supervisor and mentor at the Western Cape Government Agriculture, for his invaluable guidance, motivation, support and practical experience;

Ms R. Wentzel and the Ministry at the Western Cape Department of Agriculture, I would like to take this opportunity to say thank you, for your continuous belief in the Young Professional Persons Programme (YPP), I would also like to extend my gratitude and acknowledge how fortunate I am to be on the YPP programme and the opportunity I had to develop myself on a personal as well as on academic level.

# CHAPTER 1

# INTRODUCTION

## 1.1 Background to the research problem

A preoccupation of the 21st century is the equitable, rational and sustainable use of natural resources that underpin the worldwide food supply, because failure in this endeavour presages civil strife and starvation. The world population was predicted to reach 9.1 billion by 2050 and this will require a 70% increase in food production (Anon., 2009). Moreover, new functional food ingredients are being discovered everyday and consumer demand for “food with a healthy function” is a growing trend.

From an agricultural viewpoint, South Africa is intermediate in having both large-scale farming and significant smallholder production. It is, therefore, timely to consider how minimizing post-harvest food losses, including food waste, can help conserve resources and improve human well-being. Fermented fruit beverages not only offer a means of preservation, but also serve an important physiological function in the human diet and have been associated with lowered mortality, not only from all coronary heart diseases, but also from other diseases like cancer (Ribeiro *et al*., 2007). This was confirmed by Dias *et al*. (2007) and Duarte *et al*. (2009) who stated that the production of alcoholic fermented fruit beverages offers the potential for fruit commodities to be utilized in the food industry, which may contribute to minimizing post-harvest losses, generate more profits and promote the sustainable use of biomes. This indicates the potential of using fruits to produce fermented beverages. In countries where grapes are not abundantly available for producing wine, fermented fruit beverages are produced from local fruits that are cheap and readily available. Juice concentrates are readily storable and can be used for production processes even when the fruit is out of season. Fermented fruit beverages have not really been exploited in South Africa, except apple cider and the rest of the world. Therefore, an effort aimed at reducing the high wastage of fruits in the developing world, by investigating the possibility of exploiting underutilized fruit varieties to produce fermented fruit beverages on an industrial scale, makes good economic sense (Okunowo *et al*., 2005). Hence, it would be desirable to develop technologies to produce high value products by adding value to an underutilized agricultural produce, namely plums. It is envisaged that manufacturers who are not handling any primary agricultural produce could use these new technologies to produce fermented fruit

products from commercially available juices or pulps, hence increasing the industrial utilization of produce presently going to waste.

## 1.2 Statement of research problem

Governments are faced with the challenges of achieving local rural development in the face of globalisation in the agri-food system, liberalisation of markets, reduced state intervention and a reconsideration of the role of agriculture in rural employment and livelihoods (Bebbington, 2001). Developing countries are being encouraged to diversify their food exports by developing new products and adding more value to existing products. An analysis of production, processing, marketing channels and upgrading strategies for fresh and processed fruit with development of niche markets for high-value products create new opportunities for developing countries producers and exporters (Reardon *et al*., 2001).

Beneficial health properties of fruits have been emphasised by epidemiological studies. Evidence has pointed out how fruit utilization can play an important role in the prevention of many diseases linked to oxidative stress such as cancer, cardiovascular diseases and neuro degeneration (Dillard & Bruce German, 2000). Utilizing deciduous fruits by developing products such as fermented alcoholic beverages, provides potential access to markets for small holding and low income farmers.

Hence, there is a requirement to develop new processes for as well potential niche products from existing agricultural produce by adding more value to fruits. This could potentially lead to more production, processing and market access and new opportunities for smallholding and low income farmers, by implementing agro-processing coupled with food technology.

## 1.3 Objectives of the study

### *1.3.1 Broad objectives*

The broad objectives of this study were to measure chemical and sensorial parameters as a function of different processing conditions utilizing plums and selected berries, as well as percentage pulp levels in order to produce high quality niche alcoholic fermented beverages, as a means to highlight the importance of utilizing agricultural produce by value addition to contribute to future sustainability.

### *1.3.2 Specific objectives of the study*

The specific objectives of this study were: (1) to measure methanol, ethanol, titratable acidity, objective colour, total soluble solids and sensory profile as a function of yeast and percentage pulp in order to develop fermentation process for producing alcoholic fruit beverages using plums, an under-utilized agricultural produce; and (2) to measure methanol, ethanol, titratable acidity, objective colour, total soluble solids and sensory profile as a function of selected berries, yeast and percentage pulp with a view to produce alcoholic fermented fruit beverages based on red and white wine styles, while applying the fermentation process in the first part of the study.

## 1.4 Hypotheses

The following hypotheses formulated are non-statistical. It was hypothesized that the results would indicate the optimum combination of independent variables (percentage plum and selected berry pulp and yeast strain) in terms of the alcoholic fermented beverage in question, with regards to processing conditions as well as quality parameters.

When applying the above mentioned processing conditions to formulate white and red wine style beverages using alternative fruit varieties (plums and selected berries), it was hypothesized that sensorially acceptable niche alcoholic fermented beverages would be produced.

## 1.5 Delineation of the research

Only three varieties of plums (Songold), PR05-09 and PR04-36 (Red-fleshed plums) which are termed selections from the Agricultural Research Council South Africa and two types of berries (blueberries and blackberries) from Hillcrest Berry Orchards, Stellenbosch, South Africa were investigated. The use of more entities will escalate the amount of work far beyond the two years available for this study. The plums used in this project are seasonal, therefore preparation of all fruit pulp occurred during the 2011/2012 fruit season. The experimental fermentations were conducted at ARC Infruitec-Nietvoorbij. Fermentations were performed at cellar temperature at 15°C throughout the scientific study, since investigation of the effect of fermentation temperature on product quality was beyond the scope of this study. Minimum and maximum cellar temperature readings were recorded daily. Only one commercial enzyme preparation was used in the fermentation process. Only one commercial filtering aid, namely Kieselguhr was used.

## 1.6 Significance of the study

The significance of the research was to develop a fermented alcoholic fruit beverage with a view to increase sustainability of natural food resources (agricultural crops) by implementing agro-processing coupled with Food Technology.

## 1.7 Expected Outcomes

The expected outcome was to demonstrate ways of improving the functionality of fruit commodities by developing niche products by implementing agro-processing. This could demonstrate the potential of micro agri-industries, as well as impact on its fundamental role in employment creation and income generation. This is particularly true for the food and beverages processing sector, which remains important at all levels of economic development.

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# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Fermented beverages

The production of fermented foods is one of the oldest food processing technologies known to man. Since the dawn of civilisation, methods for the fermentation of milk, meat and vegetables have been described, with the most primitive records dating back to 6 000 BC to the civilisations of the fertile crescent in the Middle East (Fox, 1993). Records of the alcoholic fermentation of barley to beer and grapes into wine, date back about 5 000 years (Borgstrom, 1968). Over these many centuries, people developed a taste for such products that continues today in modern man. Some anthropologists (Braidwood, 1953) even suggested that it was the stimulation by and desire for ethanol that motivated man to settle down and become agriculturists. Furthermore, in ancient times, safe drinking water supplies could not be guaranteed, so the availability of fermented beverages, namely beers and wines, which kept well, was of major importance in human development. The above-mentioned fact exemplifies the original and primary purpose of fermenting food substrates, namely to achieve a preservation effect (Borgstrom, 1968).

In countries where grapes are not in abundance, wine is produced from local fruits that are cheap and readily available. Moreover, although grapes are the main raw material used for wine production, there is an increasing interest in the search for other fruits, such as apricot, apple and palm sap, which are also appropriate for wine-making. In this context, the abundance and diversity of fruits produced in South Africa has great potential to be exploited in the food industry, for example in the production of fermented fruit beverages (Dias *et al*., 2007; Duarte *et al*., 2009).

Moreover, fermented foods and beverages constitute a major portion of the diets of the majority of African people. Considerable research has been done, covering various aspects of African fermented foods and beverages (Edwards, 2003). The emphasis has been on the microorganisms used and the nutritional status of the products after fermentation. Research efforts in this field are still based on indigenous knowledge transmitted from generation to generation due to the fact that the consumers are not easily influenced by new technologies and the apparent lack of biotechnological background (Beukes *et al*., 2001). Preparations of these

products are still traditional family arts and the fermentation is by uncontrolled inoculation. Since starter cultures are normally not used, variations in the quality and stability of the products are often observed (Okafor, 1977).

### 2.1.1 Examples of fermented products in Africa

#### *2.1.1.1 Burukutu*

This is an alcoholic beverage made from malted sorghum grain, gari (a farinaceous powder obtained from cassava fermentation) and water. The mixture is fermented for two days and the end-product is a brown viscous liquid. Faparusi *et al*. (1973) reported on the microbiology of its preparation. The process includes malting and fermentation with complex mixtures of yeasts, moulds and bacteria. The pH of the final product is in the range of 3.2 − 4.0 at the end of fermentation, while the alcohol content is about 4% ($v.v^{-1}$). The beverage is popular in the Northern part of Nigeria (Faparusi *et al*., 1973).

#### *2.1.1.2 Bussaa*

Bussaa is a Kenyan opaque beer prepared from maize and malted millet. The associated microorganisms are lactic acid bacteria and yeasts. The beer contains 0.5 – 1% ($v.v^{-1}$) lactic acid, and 2 − 4% alcohol ($v.v^{-1}$) (Nout, 1980).

#### *2.1.1.3 Sorghum beer*

Sorghum beer is a traditional beverage of the indigenous people of Southern Africa and has been investigated more thoroughly than any similar product on the continent. It is an opaque, effervescent beer with a yeasty odour and fruity flavour, and the only product being produced by modern industrial processes. It is made from malted sorghum with an alcohol content of 3.2% ($v.v^{-1}$) and a lactic acid content of 0.3 − 0.6% ($v.v^{-1}$) (Haggblade & Holzapfel, 1989).

#### *2.1.1.4 Bouza*

Bouza is a fermented alcoholic wheat beverage common in Egypt. It is a thick, pale yellow drink with 3.8 − 4.0% ($v.v^{-1}$) alcohol and a pH in the range of 3.1 − 4.0. The microorganisms responsible for the fermentation are yeasts and bacteria (Morcos *et al*., 1973).

## 2.2 Value addition of underutilised crops using agro-processing as a means for economic sustainability

Agricultural intensification today is increasingly relying on a narrow range of crops (Schimdt *et al*., 2010). Of several hundred thousand known plant species, some 120 are cultivated for human food, but just nine supplies over 75 percent of global plant-derived energy intake. Of these, three species, namely wheat, rice and maize account for more than half the global energy intake (Anon, 2009). Our dependence on this relatively small number of food species raises serious concerns about the sustainability of feeding the world today and in the future (Frison, 2006; Raschke & Cheema, 2008). It is estimated that 1.2 billion people in the world do not have enough food to meet their daily requirements and a further 2 billion people are deficient in one or more micro-nutrients (Azam-Ali *et al*., 2001). Increased public awareness about underutilized species resulted from implementation of the Global plan of Action for the Conservation and sustainable Utilization of Plant Genetic Resources for Food and Agriculture (Anon, 1996). However, today their potential economic value remains “underexploited” (Padulosi & Hoeschle-Zeledon, 2004).

Faced with these pressures, many farmers are forced to innovate or find alternative strategies to remain competitive or even to survive (Katz & Boland, 2000). One of the directions many farmers consider is the development of value-added products, referred to as “the collection of activities within a company or industry resulting in the creation of a product or service valued by the consumer” (Katz & Boland, 2000). In essence, these can be raw products that farmers grow, modify and enhance by processing which significantly change the raw material and fetch a higher value (Ohmart, 2003). Value-added products derive from vegetables and fruit can be transformed into gourmet foods. Examples of these foods include sauces, vinegars, pickles, jams, spreads and preserves (Horwitz *et al*., 2008). The feasibility of utilizing underutilized fruit has been demonstrated in literature: kiwi (Souflores *et al*., 2001); caja (Dias *et al*., 2003); mango (Reddy & Reddy, 2005); and cacoa (Dias *et al*., 2007). These fruits are specifically used for producing fermented alcoholic beverages. As a result, this category of value-added products is attracting the interest of many researchers (Buhr, 2004; Kirwan, 2004; Morris & Brady, 2004). Moreover, with the maximisation of food production, including the ability to develop value-added products, becoming more vital than ever before, developed nations such as USA invested millions of dollars to assist small-growers in rural areas to add value to their produce (McConell & McGee, 2006). Thus, emphasizing the importance of

the “locality” of foods in contributing to long-term sustainability of rural communities should not be ignored. To address this need, plums, as well as selected berries have been identified as some of the important fruit commodities to be utilized in food processing and value addition (Kader, 2003).

## 2.3 Marketing trends driving technological innovation in alcoholic fermented beverages

The correlation between diet and health has increased consumer demand for more information regarding nutraceutical rich diets, with high intakes of fruits (Santos-Cervantes *et al*., 2007; Nöthlings *et al*., 2008). The bioactive constituents of berries present advantageous effects in human health and prevent chronic diseases. The World Health Organization (WHO) emphasizes the importance of antioxidant activity of phenolic components, especially from small colourful fruits, for prevention of the most important health problems namely cardiovascular diseases, diabetes, cancer and obesity (Anon. 2002; Stapleton *et al*., 2008).

Leading economists, sociologists and other intellectuals concur that the world is experiencing an era of massive and accelerating change – a change so dynamic and far-reaching that it will rearrange all facets of society over the next 20 – 30 years, rendering society virtually unrecognizable by today’s standards. Even a traditional industry such as the wine industry will not be able to evade this unavoidable metamorphosis. To navigate through the enormous challenges of the 21st century, several leading wine producing regions and countries have carried out in-depth analyses of global trends in the wine business environment and have begun to plan according to the most probable scenarios. Long-term strategies have been compiled and visions formulated to prepare the wine industries of these regions and countries for shifting consumer preferences and technological innovation. This has placed wine in the centre of the high-tension field between the forces of market pull and technology push, in which tradition and innovation need to coexist to meet the demands of wine producers and the preferences of wine consumers. The continued existence and welfare of the producer is directly dependent on the sustainable profitability of the wine industry, whereas the increasingly health-conscious and environmentally conscious wine consumer is looking for individualized, tailored products with a high ratio of quality to price. A culture of innovation permeating the entire wine industry appears to be the only route to sustainable consumer satisfaction. Therefore, it is clear that technological innovation is one of the

cornerstones with which the successful wine industries of the 21st century can be assured of winning global influence and sustainable profitability. In terms of technology, in spite of the current scepticism of some consumer groups about genetically modified organisms and products (the so-called GMO's and GM products), there is no doubt that the application of gene technology in the wine industry holds enormous potential for the future. Examples of GM technology and selective breeding applications abound in which *Saccharomyces cerevisiae* strains are currently being developed for the cost-effective, sustainable and environmentally friendly production of healthy, top quality wines that will meet changing consumer expectations (Steensels *et al*., 2014).

Another major marketing trend is looking at women as the target market (Anon. 2011). With alcohol consumption amongst women increasing, leading brands and manufacturers are targeting women more specifically within the alcoholic beverage sector. Testimony to this is the fact that, of new products launched in this sector, those aimed specifically at women is double that compared to those aimed specifically at men (Sadler, 2005). Hence, it is clear that more brands are targeting women, through new product development and marketing (Anon, 2007). Three world-renowned beer manufacturers recently used new product development and marketing strategies to capitalize on this gap in the market (Anon, 2007; Anon, 2011). In terms of formulating products that will appeal to the female palate, Heineken and Carlsberg introduced "Wieckse Rose" beer and "Rooted in Copenhagen", respectively. The latter is described as light and refreshing with a relatively low alcohol content of 4.5% and is brewed from natural ingredients (Anon, 2011). SABMiller introduced a new brand called "Redds" that is aimed specifically at women. This product is an apple-infused malt beverage with rich fruity flavour. With sales to women in Europe, Latin America and South Africa at 70 to 80%, this product clearly appeals to the target market (Anon, 2007). Marketing strategies include product names that appeal to women (e.g. "Eve") (Anon, 2011), aesthetic appeal in terms of packaging and appearance (Anon, 2011) and advertising in magazines directed at women (Anon, 2007). It even extends to multiple packs shaped like a woman's handbag with only 5 − 10 bottles per pack to further ensure ease of handling by women (Anon, 2007).

## 2.4 Plums and its potential to be utilized for value addition

Plums constitute the most numerous and diverse group of fruit tree species. The immense variety of plums, the distribution of the fruit through a wide area, and its adaptability to varying conditions make it of great importance not only at present, but also for future utilization and exploitation (Blažek, 2007). The latter is linked to the potential of plums to contribute greatly to human nutrition because of their richness in fibre and antioxidants (Stacewicz-Sapuntzakis *et al*., 2001; Kim *et al*., 2003). Neochlorogenic acid and chlorogenic acid are the two dominant phenolic compounds in plums (Donovan *et al*., 1998), while individual phenolics showed characteristic antioxidant capacities (Heo *et al*., 2007). In addition, consuming peaches, plums and nectarines is positively associated with nutrient intake, improved anthropometric measurements and reduced risk of hypertension (Beals *et al*., 2005).

Plums are an important crop economically, because of the export revenue. South Africa is a major plum exporting country with annual export figures (2009) of close to 9 million cartons (5.25 kg equivalent cartons) comprising 35 different plum cultivars and an annual supply window of six months (October to April), with shipping mostly to markets in the European Union and the United Kingdom (Anon., 2011). Plums sold on the export markets generate a greater unit price than that achieved on the local market. In terms of processing, the volumes of plums available for processing in South Africa fluctuate yearly, depending on the crop size and the percentages of exportable fruit. In 2009 – 2010, the processing industries absorbed approximately 2% of all plums produced. Moreover, the production often goes to waste due to poor market demand and limited processed product lines (Figure 2.1). This results in large volumes of plums rotting in plum orchards due to lack of utilization, leading to enormous waste during the production season (Bhutani & Joshi, 1995). Therefore, this clearly indicates that formal strategies in terms of processing plums are required. As a result, food scientists and technologists have demonstrated the potential need to develop various niche value-added products to enhance economical sustainability.

### *2.4.1 Alternative uses of plums in the food industry*

According to Decker (1999), plum-derived food ingredients have been reported to function as antioxidants, antimicrobials, fat replacers, and flavourants. Hamburgers

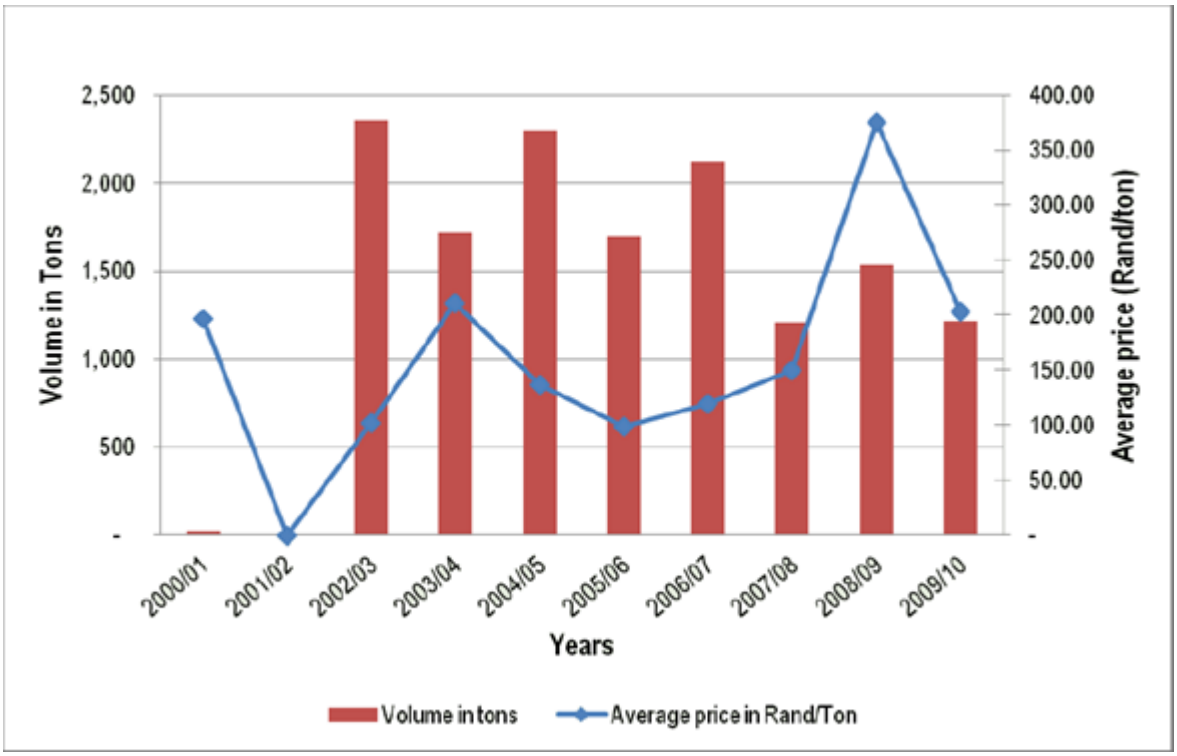


**Figure 2.1** The amount of plums purchased for processing for the past decade in South Africa (Anon., 2011)

containing dried plum puree has been reported to retain 15.8% more moisture when reheated and held for up to 4 h. Moreover, dried plum puree at addition levels of 3% and higher has been shown to be as effective as butylatedhydroxylanisole (BHA) and butylatedhydroxytoluene (BHT) which are synthetic antioxidants responsible for retarding lipid oxidation in precooked pork patties (Nuñez de Gonzalez *et al*., 2008). Similarly, according to Lee & Ahn (2005), plum extract used at >2% in turkey breast rolls and irradiated at 3.0 kGy was effective at retarding lipid oxidation while enhancing juiciness. These studies indicate that dried or fresh plum products might also serve to protect flavour in precooked, whole muscle meats injected with a brine marinade. According to Dauter *et al*. (1999), the addition of plums improves the rheological, physical and chemical properties of bread by increasing vitamins A, B and C, magnesium, sodium and especially potassium. As a result an innovative range of bread with high energy and vitamin value is obtained.

Juices are economically very important fruit products. Among commercially available juices orange juice is the most popular, followed by apple juice, while plum juices are very rare. Three main groups of juices are known: clear, cloudy and pulpy juices. The processes of juice separation from fruit cells are different based on the type of juice desired and for the first two juice types, separation is achieved by pressing. All fruits are not suitable for all type of juices, e.g. fruit with the colour pigments insoluble in water is not suitable for producing clear juices. The red colour of plum skin originates from anthocyanins, pigments that are soluble in water, making it suitable for all types of juices (Lovrić, 1984). Many authors have studied the usage of pectolytic enzymes in juice production with a view to improved yield and colour extraction *inter alia* in plum juices (Will & Dietrich, 2006). Their effectiveness depends on origin of enzyme and treatment conditions (pectolytic enzyme dose, maceration duration and reaction temperature) (Kashyap *et al*., 2001). The purpose of adding pectolytic enzymes is degradation of proto-pectin and partial pectins from primary cell walls and middle lamellae and this process is responsible for the release of juice from the cells as well as the pigments from the plum skin cells (Will & Dietrich, 2006). Pectin should not be degraded completely because it stabilizes the cloudiness in cloudy juice. Further value addition of plum juices can be achieved by fermentation technology. Numerous studies have been used for the preparation of alcoholic beverages from plums which includes wine, sparkling wine and brandy styles (Tesevic *et al*., 2005; Bhardwaj *et al*., 2005). However, the objective of using plums for wine-making has not been completely realized in South Africa, though wine and

brandy is prepared commercially from this fruit in numerous countries of the globe. Production of plum wines with high phenolic compound content would be of particular “health” interest when it is seen as one of the less costly raw materials amongst the fruits. Like plums, various berries are suitable as raw material for wine-making.

## 2.5 The potential of utilizing selected berries

Rubus fruit have long been collected and consumed worldwide (Finn, 2008; Hummer, 2010), regardless of whether they were recognised for their possible health benefits from their natural phytochemicals or merely because they tasted good (Rao & Snyder, 2010). Rubus also has a pharmacological history, which was reviewed by Hummer (2010). Today, Rubus fruit are considered a healthy and nutritious food, containing phenolics, vitamin C (Borges *et al*., 2010), dietary fibre (Acosta-Montoya *et al*., 2010), calcium (Plessi *et al*., 2007), potassium, magnesium, carotenoids (Mertz *et al*., 2009), linoleic acid and linolenic acid (Kim *et al*., 2011).

The global attractiveness of Rubus fruit has increased in part due to the ongoing published accounts of highly coloured berries and other fruit and their potential health benefits (Ross *et al*., 2007; Seeram, 2008; Rao & Snyder, 2010). Since they have a broad diversity of phenolic compounds (Szajdek & Borowska, 2008; Nurmi *et al*., 2009), blackberry (*Rubus sp*.), blueberry (*Vaccinium. corymbosum*), blackcurrant (*Rubusrugrum*), cranberry (*Vacciniummacrocarpon*), raspberry (*Rubusideaus*) and strawberry (*Fragariaananassa*) are more often than not consumed in fresh or processed forms in the human diet. Berries are remarkably rich sources of antioxidant phenolics (Seeram *et al*., 2008). Their activity is manifested by the scavenging ability of reactive oxygen species, such as hydroxyl, peroxide radicals and radicals of other reactive forms of oxygen, including hydrogen peroxide and singlet oxygen. As a result these radicals inhibit the activity of enzymes and form complexes with metals which catalyze oxidation reactions (Heim *et al*., 2002).

The aforementioned phenolic compounds are the most important group of phytochemicals in berry fruits and include flavonoids (anthocyanins, flavonols, flavones, flavanones and iso-flavonoids), stilbenes, tannins and phenolic acids. Many phenolic compounds of berries are responsible for the colour (anthocyanins) and flavour (tannins). The range of phenolic compounds in berry fruits is determined by many factors, such as species, variety, cultivation, region, weather conditions, ripeness, harvesting time, storage time and conditions (Skupieñ & Oszmiañski, 2004; Anttonen & Karjalainen, 2005; Ehala *et al*., 2005; Castrejón *et al*., 2008). Some

factors such as species, variety, geographic region, storage conditions, ripeness, climate and others may also influence the concentration of the phenolics in berries (Benvenuti *et al*., 2004). The phenolic compounds protect plants against undesirable factors such as infections, physical damage, UV radiation and other factors.

## 2.6 Winemaking process

Wine is a beverage resulting from the fermentation of grape juice by yeasts with appropriate processing and additions (Mateo & Maicas, 2016). All wines are made using a common process, with variations depending on the type to be produced (Figure 2.2). As soon as the grapes are harvested, they are transferred to the winery where they are crushed and stemmed resulting in the must (Vine *et al*., 1997). Depending on the type of wine, the juice may be separated from the skins and is treated with sulphur dioxide in order to prevent oxidation or the growth of spoilage microorganisms. Fermentation, which is the most significant stage of vinification, is aided by yeast being added to convert the sugars to ethanol at different stages (Boulton *et al*., 1996). Fermentation takes place in tanks and it takes from 5 to 14 days. After fermentation, the wine is drawn off to separate it from the sediment of largely dead yeasts. The suspended particles must be removed by clarification. Wine is either bottled after clarification or after ageing in wooden containers made of oak. The wood aging process may last many months or several years. Before bottling, wine may require blending, clarification or filtration (Mateo & Maicas, 2016).

## 2.7 Fermentation process and yeasts interaction

The fermentation process for the development of a fermented fruit beverage depends on the ability of yeast to convert sugars into alcohol, esters and other volatile and non-volatile compounds. Due to the differences in fruit composition, yeast strains used for fermentation have to adapt to a different environment in terms of sugar composition and concentrations and the presence of organic acids (Duarte *et al*., 2009). *Saccharomyces cerevisiae* is the main yeast used in winemaking, due to its high fermentation capacity. The impact of yeast strains on fermentation vary. According to Colombie *et al*. (2005), only moderate differences fermentation efficiency were observed when comparing 20 randomly chosen commercial strains of *S. cerevisiae* (i.e. without taking their fermentative capabilities into account) cultured in an easily fermented synthetic medium.

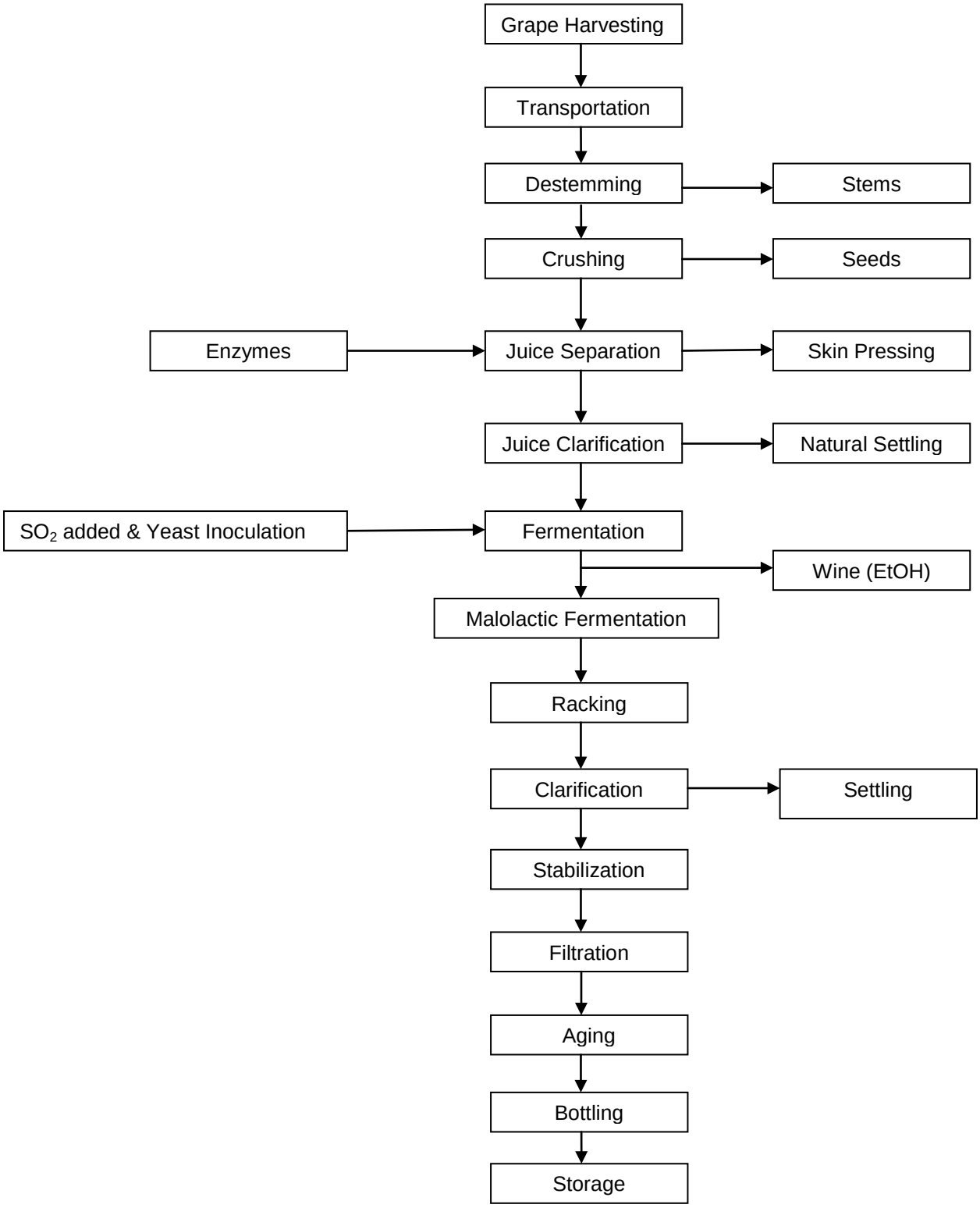


**Figure 2.2** Schematic diagram of general wine-making process (Soufleros, 1997)

Much larger differences were reported by Blateyron & Sablayrolles (2001), in a comparison of 13 randomly chosen strains cultured in a difficult-to-ferment must (natural must leading to sluggish or stuck fermentations): three strains resulted in stuck fermentations, with residual sugar contents of 10 – 56 g $L^{-1}$, whereas the remaining 10 strains fermented all the sugar, with a fermentation duration of 119 – 170 h. Therefore, the choice of strain used by the winemaker is increasingly motivated by the potential impact of that strain on the wine characteristics, whilst considering the availability of the large number of existing strains, the many complex mechanisms of interaction between yeast, must and the fermentation conditions. Specific strains are now widely acknowledged to be valuable for: (i) increasing the fruity character; (ii) improving some varietal characters such as in Sauvignon (Dubourdieu *et al*., 2006) and Chardonnay wines (Eglinton *et al*., 2000); (iii) increasing the expression of varietal characters by the hydrolysis of glycoside-bound volatile compounds during fermentation (Ugli-ano *et al*., 2006); (iv) limiting the production of organic acids or increasing the production of glycerol (Scanes *et al*., 1998); (v) limiting off-flavours, including those due to sulphur (Rauhut, 1993) and volatile phenols (Shinohara *et al*., 2000); (vi) the value of specific strains for producing mannoproteins (Moine-Ledoux & Dubourdieu, 2002); and (vii) for improving the colour of red wines through their interactions with polyphenolic compounds (Medina *et al*., 2005).

## 2.8 Factors affecting yeast growth

Wine is a natural product resulting from a number of biochemical reactions. Many of these reactions are attributed to nature and the microorganisms present (Torija *et al*., 2001). Apart from the main wine yeast, *S. cerevisiae*, spontaneous alcoholic fermentation of must is an intricate process carried out by the sequential action of different yeast genera and species (Heard & Fleet, 1988).

Grape must is usually fermented by *S. cerevisiae* strains, which is the yeast mainly being responsible for the quality and flavour of the final product (Pretorius, 2000). The nutritional needs of *S. cerevisiae* species to produce wines with desirable organoleptic characteristics are relatively high, and many factors have been found to influence their growth and their metabolic capabilities, including sugar content, temperature, aeration and nitrogen availability (Bisson, 1999; D'Amato *et al*., 2006).

Sugar content is one of the most important factors that influence microbial growth during wine fermentation. Grape must usually contain equal amounts of

glucose and fructose (Fleet & Heard, 1993), but in some ecological conditions and grape varieties, the proportion may differ. As a consequence of the global climatic change, fructose concentration in grapes is increasing relative to glucose, affecting wine quality globally (Jones & Jew, 2007). Although glucose and fructose are co-consumed by yeasts during wine fermentation, *Saccharomyces* strains have a preference for glucose, which is usually consumed faster, resulting in a reduction in the glucose/fructose ratio, and the preponderance of fructose towards the end of the fermentation (Berthels *et al*., 2004). However, it has been shown that this preference for glucose over fructose varies among strains and is dependent on the yeast's genetic composition and on external conditions (Beltran *et al*., 2005; Messias *et al*., 2008). A high sugar concentration at the beginning of the fermentation process and high amounts of ethanol at the end, subject yeast cells to varying degrees of osmotic and ethanol stress. That often leads to cessation of fermentation, termed a stuck fermentation. There is also considerable variability among yeasts depending on the species and the strain and the conditioning of the yeast to grow at high sugar concentrations (Reed, 1982). As a result, alcohol production can be lower in a must containing 300 g $L^{-1}$ of sugar than in must containing only 200 g $L^{-1}$ of sugar. At sugar levels beyond 350 g $L^{-1}$, the must becomes practically non-fermentable. Therefore, elevated amounts of sugar hinder yeast growth and decreases the maximum population. Consequently, fermentation slows and becomes stuck even before a significant quantity of ethanol is produced (Ribéreau-Gayon *et al*., 2000).

Nitrogen is an important macronutrient that plays a major role in many biological functions of fermentative microorganisms (yeast and malolactic bacteria). Yeast growth, fermentation kinetics and flavour metabolism are all greatly affected by the nitrogen status of the must (Henscke & Jiranek, 1993). During wine-making conditions, initial low levels of nitrogen limit growth and biomass, resulting in a reduced fermentation rate. Assimilable nitrogen content is another important factor that directly affects the course of fermentation. During the phase of fermentation when nitrogen sources are consumed and ethanol concentrations are high, some strains have difficulty to ferment the remaining fructose, resulting in slow (sluggish) and incomplete (stuck) fermentations (Bauer & Pretorius, 2000). Hence, nitrogen deficiencies has been associated with major problems encountered in contemporary wine-making, especially those related to sluggish and stuck fermentations (Bisson, 1999; Varela *et al*., 2005). Since nitrogen is such an important macronutrient, with a major role in many of the functions and processes carried out by the yeasts, the

intrinsic importance of nitrogen content on both yeast growth and its metabolism is well known to winemakers. A minimum concentration of 140 mg $L^{-1}$ is often quoted as necessary for the fermentation of a must with a moderate sugar content of 200 $gL^{-1}$ (Bell & Henscke, 2005).

## 2.9 Factors influencing various stages of winemaking

Oxygen is introduced, in an uncontrolled way, at various stages of winemaking, especially at pressing, during pumping over and other wine transfer operations, culminating at bottling (Castellari *et al*., 2004). It is widely accepted that oxygen ($O_2$) contributes to wine character by impacting on colour, aroma and mouth-feel properties due to oxidation. Oxidation is the process where electron transfer takes place between reductive and oxidative partners. In fermented beverages, oxygen is predominantly responsible for oxidation reactions, thereby reducing certain compounds to intermediates and eventually to hydrogen peroxide and then water (Danilewicz, 2003). During the production process, fermented beverages come into contact with air which inevitably results in different $O_2$ concentrations dissolving in the fermented beverage. For example, the must can be almost saturated with $O_2$ during crushing and pressing of fruits (Schneider, 1998). Subsequent operations such as pumping, transfer from tank to tank, filtration, racking, centrifugation, bottling and aging add more $O_2$ to the fermented beverage (Vivas *et al*., 2003). When fermented beverages are saturated with $O_2$, it contains about 6 – 8 mg $L^{-1}$ $O_2$ at cellar temperatures. Fermented beverages are, however, seldom saturated with $O_2$, due to insufficient contact of air during the production process. Temperature also influences the dissolved $O_2$ saturation level, with $O_2$ concentrations higher at lower temperatures (Vivas de Gaulejac *et al*., 2001).

Contact of fermented beverages with $O_2$ can be minimized by the use of inert gases, such as $N_2$, $CO_2$ and even argon gas. The addition of $SO_2$ can also influence the rate of $O_2$ consumption due to the fact that $SO_2$ predominantly has a direct anti-oxidative effect and also inhibits oxidation enzymes. However, in fermented beverages, chemical oxidation occurs and mainly the sulphite forms of $SO_2$ may react with $O_2$, but these reactions are slow under fermented fruit beverage processing conditions such as low pH and high ethanol levels (Ribereau-Gayon, 2000).

Oxygen addition, which generally occurs to improve biomass synthesis and therefore to increase the fermentation rate, has been well-studied during oenological

fermentations (Sablayrolles *et al*., 1996) and has been found that oxygen addition is efficient only at the end of the cell growth phase (Sablayrolles *et al*., 1996). A recent study showed that lipid synthesis and optimal growth of *S. cerevisiae* during alcoholic fermentation required about 5 – 7.5 mg of oxygen $L^{-1}$ (Rosenfeld *et al*., 2003). These values are consistent with previous data obtained under oenological conditions namely 5 – 7 mg $L^{-1}$ (Sablayrolles & Barre, 1986). However, during cider-making (O'Connor-Cox *et al*., 1993), lower requirements of oxygen (between 1.5 and 2 mg $L^{-1}$) were found. The absence of respiratory chain activity during winemaking may be due to the very high sugar concentrations, which repress respiratory chain activity and, to a lesser extent, fatty acid desaturation activities (Salmon *et al*., 1998). These differences may also be due to the oxygen consumption by yeast cells during alcoholic fermentations being attributed by several authors to mitochondrial respiration only (O'Connor-Cox *et al*., 1996; Dinsdale *et al*., 1999).

The methanol content is also of major importance when producing fermented fruit beverages. The increase in methanol in fermented fruit beverages is often associated with activities of pectin methyl esterase (PME) and pectatelyase (PAL) enzymes present in fruit juices (Hou *et al*., 2008). The addition of commercial pectic enzymes (CPE) plays an important role in the process of making fermented fruit beverages, where it assists with extraction, clarification and filtration of fruit juice and wine puree to increase the yield and quality (e.g. pigment, flavour, clarity, and viscosity) (Soufleros *et al*., 2002). Hence, methanol is produced in large quantities after enzymatic degradation has taken place during fermentation and the aging period of the fermented fruit beverage (Wu *et al*., 2007). As a result, using CPE in the fermentation stage of fermented fruit beverages could be a major drawback if it results in methanol levels that exceed the regulatory safety limit stipulated for some wine products. To overcome the increase in methanol during the fermentation stage, Hou *et al*. (2008) added gallic acid or coumaric acid during wine-making, resulting in a decrease in the methanol content.

The range of temperatures used for winemaking fermentations is quite large, from 15°C for white wines to more than 30°C for red wines. Furthermore, most wine fermentations are not run at constant temperature. In most cases, the final temperature is controlled, but is preceded by a phase in which heating due to fermentation (23.5 kCal per mole of sugar) is not compensated by cooling, and the difference between initial and final temperature may exceed 10°C (Sablayrolles & Barre,1993). Low temperatures increase the production of volatile compounds

(esters, acetates and medium-chain fatty acids) by the yeast during the alcoholic fermentation (Torija *et al*., 2002). Hence, such temperatures (10 – 15°C) may be used by winemakers to enhance the production of these volatile compounds, improving the aromatic profile of the wine.

## 2.10 Sensory attributes and sensory evaluation of fermented fruit beverages

Major contributions to the sensory attributes of wine come from compounds originating from grapes and other fruits and also from yeast and bacterial metabolism during alcoholic fermentations (Swiegers *et al*., 2005). The complexity of the system is increased by the fact that biological transformation of compounds originating from grapes and other fruits may occur due to microbial activity during fermentation and that chemical transformation may occur in the acidic conditions found in wine (Francis & Newton, 2005).

Wine flavour and aroma are complex mixtures that are derived from multiple sources during vinification. These wine flavours and aroma compounds are determined by the relative concentration of compounds from various sources (Torrens *et al*., 2008), variables that are introduced during winemaking and are influenced by the volatile composition present in wine. For example, the uses of various types of yeast strains as well as malolactic bacteria have been shown to alter sensory properties of wine (Bartowsky, 2005).

A typical alcoholic fruit beverage is a complex product with sensory qualities attributable to the variety of fruit, fermentation conditions, which include yeast strain variation and other vinification aspects, such as barrel or bottle ageing. These sensory attributes are critical to the overall quality of the alcoholic beverage and have been widely examined and characterised (Gawel *et al*., 2001; Pickering *et al*., 2008).

With regards to the sensory attributes of wine, according to Nykanen (1986) flavour is the most important sensory parameter evaluated to determine character and quality of alcoholic beverages. Flavour has two types of components, those giving rise to the taste sensation and those responsible for the aromatic character (Williams & Ismail, 1981). Taste components include non-volatile chemicals such as sugars, acids, salts and polyphenolic material that give rise to taste sensations such as astringent and bitter. The sweet taste in wine is primarily elicited by glucose and fructose and could be enhanced by ethanol and glycerol (Thorngate, 1997). The sour taste (acidity) is induced by organic acids present in wine and is influenced by the

pH. Tartaric acid is responsible for more than half of the acidity (Thorngate, 1997), but malic and other acids may also be present (Grab, 2007). The salty taste is associated mostly with inorganic cations and anions (Jackson & Lombard, 1993). Bitter tastes in wine could be elicited by structurally diverse phenols and polyphenols, but is often confused or masked by the astringency sensation. Tannin monomers are the most intensely bitter compounds, while ions and amino acids can also produce bitterness (Noble, 1998).

Aroma volatiles, on the other hand, create that characteristic aroma of a fruit and include some key compounds distinguishing one fruit from another (Grab, 2007). It is often not a single compound that represents a characteristic fruit flavour, but rather a combination of compounds working in synergy (Schotsmans & Prange, 2006). These are often referred to as “character impacting compounds”. Yeast strains have an effect on flavour, including aroma (Subileau *et al*., 2008). The most pronounced changes in flavour often take place during the fermentation and result in changing the aroma profile of the fruit juice. The major cause of this is the production of yeast volatiles and the metabolism of original fruit volatiles. Volatiles from yeast metabolism also result in the subsequent formation of esters which are important contributors to wine flavour (Cabaroglu *et al.,* 2005).

Aroma compounds are especially important in fermented fruit beverages as they contribute to the quality of the final product. Wine or other fermented fruit beverage aroma is extremely complex, due to the great number of compounds present. These may have different polarities and volatility and may be found in a broad array of concentrations (Etiévant, 1991).

From an organoleptic point of view, the two main bioprocesses are the alcoholic fermentation and the malolactic fermentation (Herrero *et al*., 1999). During the alcoholic fermentation, yeast (*S. cerevisiae*) transforms the majority of sugars (fructose, glucose and sucrose) into ethanol and $CO_2$ by the Embden-Meyerhof-Parnas pathway (Williams, 1974). This is the fundamental bio-reaction, but it is not the only one and hence numerous organoleptic products are also formed. As with the aroma profile, the alcohol profile may be found in a wide range of concentrations (Etiévant, 1991). The alcohol profile is a significant factor in the quality of wines (Anuna & Akpapunam, 1995). Though the fermentation of fruit sugar usually yields ethanol as the predominant alcohol, small quantities of higher alcohols are also produced from the oxidative deamination, decarboxylation and reduction of amino acids and sugar degradation (Anuna & Akpapunam, 1995). The presence of pectin in

some fruits may also result in methanol generation in the fermenting juice (Anuna & Akpapunam, 1995). Since both the aroma and alcohol profile depend on the composition of the juice and on the activity of the yeast, the quality of wine produced greatly depends on the yeast strain (Okunowo *et al*., 2005).

As mentioned, another important transformation during wine-making is the malolactic fermentation (Figure 2.3). It is an optional secondary fermentation that uses lactic acid bacteria (LAB) to metabolize harsh tasting malic acid into a softer more rounded acid, namely lactic acid (Browning *et al*., 1997).

The most important of the sensorial compounds are the organic acids, the higher alcohols and the esters. Most of these esters are formed at the beginning of the fermentation and decrease or remain stable towards the end of the fermentation. Quantitatively, the main ester is ethyl acetate derived from the ethanolysis of acetyl Co-A (Williams, 1974). In contrast to ethyl esters of higher molecular weight alcohols, which are desirable elements of the aroma of wines, ethyl acetate confers a disagreeable odour. Amerine *et al*. (1972) reported that an ethyl acetate concentration over 200 mg $L^{-1}$ negatively influences wine quality. The biosynthesis of esters is affected by several factors, such as the aeration of the must, the fermentation temperature, the technique of fermentation and the maturity of the fruits. It is, therefore, important to control the fermentation to ensure the optimum profile of these volatile compounds.

Glycerol is the major and the most important non-volatile compound produced by yeasts in wines and significantly contributes to the wine quality by providing slight sweetness and fullness. In other words, it is considered as the third major compound produced during wine fermentation after ethanol and carbon dioxide. The amount of glycerol formed during fermentation by *S. cerevisiae* is around one-tenth of the amount of ethanol produced. As a result, its concentrations in wine varies between 1 − 10 g $L^{-1}$ (Ough *et al*., 1972), although normal concentrations are in the range 4 – 9 g $L^{-1}$. Like ethanol production, glycerol production by yeast is affected by many growth and environmental factors (Gardner *et al*., 1993; Remize *et al*., 2000).

Characterising sensory attributes of alcoholic beverages is generally done by a tasting panel that assess each alcoholic beverage individually for a number of attributes. Sensory evaluation (SE) is a scientific method used to evoke, measure, analyse and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing (Stone & Sidel, 1993). SE can be divided into

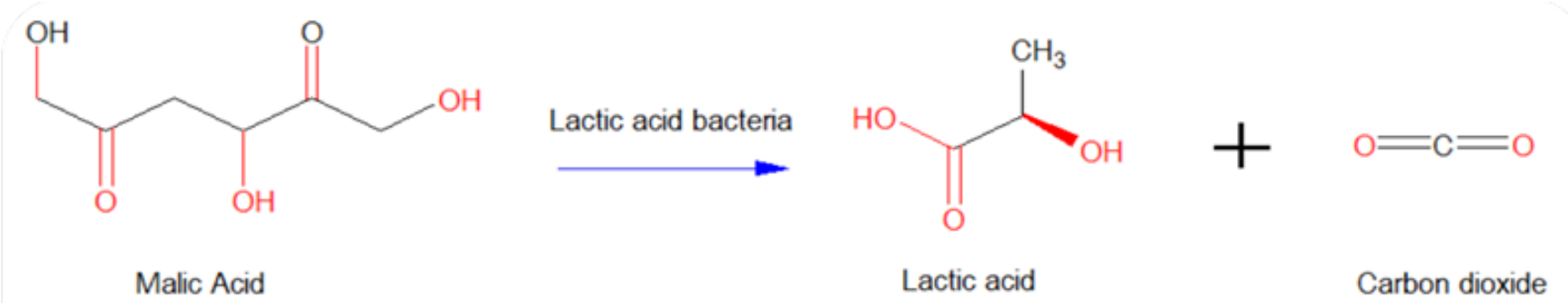


**Figure 2.3** Metabolism of malic acid by Lactic acid bacteria (Browning *et al*., 1997)

two categories, objective (analytical) and subjective (affective). In objective testing, the sensory attributes of the product is evaluated by a selected trained panel, while in subjective testing, the reactions of consumers to the sensory properties of the products is measured.

A class of analytical SE techniques often used for beverages is descriptive analytical methods which enable the scientist to obtain complete sensory descriptions. These descriptions are always suited to the type of product being developed to acquire the attributes which are essential to the acceptance thereof. In beverage products this type of analyses can be a critical tool to identify quality defects, i.e. it is used to investigate processing problems (Lawless & Heymann, 1998). According to Lawless (1999), descriptive analysis is the primary sensory technique for analysing complex aromas, fragrances and flavours. The use of a trained panel to measure the intensities of specific attributes is the foundation of this type of analysis. The main objective of panellists is to provide an intensity rating for each of the attributes of the product in question that reflects the perceived intensity of that specific characteristic or attribute in the product. This technique often involves the training of the panellist to score the respective samples in terms of the specific sensory attributes on a line scale; the determination of panellist reproducibility of each panellist; analysis of the data according to an experimental design specific to the study; followed by analysis of variance or an appropriate multivariate statistical technique (Lawless & Heymann, 1998).

Another class of methods of sensory analysis are those based on consumer acceptance and preferences. Consumer sensory analysis is often performed at the end of product development or of a reformulation cycle. These methods are frequently used to compare prototypes or market competitors. In conducting these sensory tests, two main approaches are usually followed, namely the measurement of preference or the measurement of acceptance (Lawless, 1999). In measuring preference, the consumer has a choice between competing products and has to choose one product over another. In measuring acceptance or liking, consumer panellists often rate their liking on a scale known as the 9-point hedonic scale, which measures the degree of liking, i.e. preference, as well as the level of acceptance. According to Lawless & Heymann (1998), acceptance tests can be performed on one product, but multi-product tests are also common. Preference can be determined indirectly from the hedonic scores since each unit on the scale is anchored by a “degree of liking” description. For example, in terms of mouth-feel, the panel will evaluate descriptors such as body, warmth, astringency, while taste will include sourness, bitterness and sweetness. When consuming an alcoholic beverage, an important mouthfeel sensation

perceived during consumption is astringency. According to Noble (2002), it is believed to be perceived by touch *via* mechanoreceptors.

## 2.11 Factors affecting quality and spoilage of wine

Unlike industrial alcoholic fermentations, wine fermentations do not aim to maximise the concentration or yield of a defined metabolite, or the productivity of the process. In winemaking, the main objective is to optimise product quality, which is very difficult to quantify. Wine tasting remains the best way to assess the characteristics of wine, but is difficult, imprecise and time-consuming. The control of technological parameters, such as sugar consumption, the duration of the fermentation and the amount of energy required to regulate fermentation temperature, is also of interest. Many studies have shown that fast fermentations may be detrimental to wine quality, especially for white wines. On the other hand, too long a fermentation both delays the subsequent processes and increases the risks of wine spoilage. Control of fermentation kinetics is generally considered as a prerequisite for controlling the characteristics of the wine (Swiegers & Pretorius, 2007).

The main role of micro-organisms in winemaking is to convert sugars to alcohol, reduce wine acidity and introduce interesting and desirable aroma and flavours to wine. Hence, in many cases microbial spoilage is not easily defined, particularly in fermented alcoholic beverages, where the concept of spoilage yeast has a more complex meaning, since any yeast has the potential to change sensorial characteristics and can be regarded as either beneficial or as a ‘‘spoilage yeast’’ (Fleet, 1992). Detrimental and beneficial activity must, therefore, be distinguished. Since microbiological activity can develop quickly and without warning, early identification of potential spoilage problems is of high importance in winemaking. Identifying the causative microorganisms is not always simple because a given microorganism can bring about multiple spoilage problems. Spoilage microorganisms mainly include yeasts of the genera *Dekkera, Brettanomyces, Candida, Hanseniaspora, Pichia, Metschnikowia, Saccharomycodes, Schizosaccharomyces* and *Zygosaccharomyces* (Enrique *et al*., 2007), lactic acid bacteria and acetic acid bacteria (Luoreiro & Malfeito, 2003). Many detrimental effects of yeasts occur before fermentation, e.g. ethyl acetate produced by *Pichi aanomala* (Plata *et al*., 2003) or during the early stage of fermentation, e.g. acetate production by *Kloeckera apiculata and Hanseniaspora uvarum* (Romano *et al*., 1992). Other common spoilage effects are film formation in stored wines, cloudiness or haziness, sediments and off-tastes (Du Toit & Pretorius, 2000; Loureiro & Malfeito-Ferreira, 2003).

These effects are the results of activity by bacteria and yeasts (Fleet, 2003). In fact, most traditional wine ‘‘diseases’’ are bacterial in origin (Ribereau-Gayon *et al*., 2000).

Based on the discussions in previous sections it is clear that there is great dissimilarity of composition between fruits. Hence, to produce these beverages there is a necessity for more studies in terms of the ideal yeast strain, fermentation temperature and the type of must treatment, or treatment of the fruit pulp, during the pre-fermentative phase and during the fermentation (Jennifer, 1999).

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# CHAPTER 3

# THE DEVELOPMENT OF ALCOHOLIC FERMENTED BEVERAGES UTILIZING PLUMS

## 3.1 Abstract

The adaptation of using existing technologies toward producing an alcoholic fermented plum beverage based on white wine style was undertaken in this study. The Independent variables (ID) were yeast strains (1) *Saccharomyces cerevisiae* VIN13 and (2) *Saccharomyces bayanus* N96, with formulations containing percentage pulp concentrations at (40%, 50% and 60%). The dependent variables (DV) constituted key quality parameters for white wines, namely methanol, ethanol, titratable acidity, objective colour, total soluble solids, pH and sensory profile were measured. The titratable acidity (TA) increased as the concentration of pulp increased in formulations containing 40 – 60%. The pH range of alcoholic fermented plum beverages was similar to that of white wines produced in South Africa. The %ethanol ($v.v^{-1}$) ranged between 11.60 – 11.99%, which was slightly higher than the target %ethanol based on the beverage formulations developed in this study, namely 10% ethanol ($v.v^{-1}$), but was within the typical range for white wines. Differences in %EtOH amongst treatments were found to be not significant ($p > 0.05$). Methanol was not detected in the samples. Objective colour measured in the alcoholic fermented plum beverages was also similar to objective colour measured in white wine, but with a more intense “yellow” colour. The overall sensorial profile of the alcoholic fermented plum beverages, fruity aroma and sweet associated aroma were rated significantly higher as the pulp concentration increased from 40% to 50% ($p < 0.05$). Fruity flavour at 60% pulp concentration was rated the most intense. The differences in sweet taste between samples were not significant ($p > 0.05$). This agreed with the sugar levels that were measured in $^{\circ}$Brix which were all very similar and not significantly different ($p > 0.05$). Sour taste was rated significantly lower ($p < 0.05$) at 40% pulp concentration, than at 50% and 60%, while the panel rated the sour taste most intense in the majority of the samples containing 50% pulp. The results partially agreed with the results for titratable acidity (TA) with the lowest TA recorded for 40% pulp. However, the highest TA was measured for 60% and not 50% pulp. The pH measured for all pulp concentrations were similar. Bitter taste was rated

significantly different ($p < 0.05$) for 40% pulp concentration samples. However, the results showed inconsistency in the panel responses as no clear trend was identifiable, in some cases the bitterness in 50% pulp concentration samples were rated most prominent, whilst in other cases the 40% pulp concentration samples were rated most bitter. Therefore, the influence of astringency in samples could have confounded the observation of the panel's response to bitterness (Noble, 1999). The outcome of the study showed that adapting existing technology can be used to produce an alcoholic fermented plum beverage of which the key quality parameters and attributes are comparable to white wines.

## 3.2 Introduction

The South African plum fruit industry is well established and for the most part focused on supplying plums to the export market. The majority of South African plums are exported to northern hemisphere countries for the duration of their winter and spring seasons (Anon., 2011). South Africa's major plum producing areas situated in the Western Cape Province are the Little Karoo, Paarl, Wolseley/Tulbagh and Stellenbosch areas. The aforementioned areas account for more than half of the plum production, making the Western Cape the leader in plum production. This is largely owing to the favourable weather conditions and the Mediterranean type climate (cold winters and hot, dry summers) (Anon., 2011). However, inspite of the abundant availability of this crop locally, very low volumes are processed or preserved. According to the Department of Agriculture Forestry and Fisheries (DAFF) only 2% of all plums produced in South Africa are utilized by the processing industry (Anon., 2011). This indicates the potential to utilize plums to a greater extent to create value-added niche commodities. Successful expansion of the marketability of underutilized plums could contribute to efficient risk management, enhancing the stability of farming systems and enhance local empowerment (Jaenicke & Lengkeek, 2008). Therefore, many organisations from both the government and non-government sectors are actively promoting the processing of fruit, since fresh produce largely go to waste due to difficulties in effectively handling seasonal gluts. The handling problems include insufficient capacity to store large quantities of fresh produce without incurring heavy losses, local markets that are too small for the large quantities of fresh produce in season and ineffective distribution and transportation to meet the demand in other areas (e.g. urban areas) (Gòmez & Ricketts, 2013). Due to these constraints, rural producers are often forced to give produce away or let it rot.

To prevent this loss, attention is drawn towards converting such gluts into value-added products to be sold commercially in the retail market.

Processing of fruits into value-added products is the best alternative to control the huge losses. Processed fruit products generally include minimally processed fruit products such as fresh-cut fruit, fermented fruit products such as cider, wine and vinegar, traditional thermally processed fruit products such as jam, jelly, juice and beverages, novel non-thermal processed fruit products such as juice and beverages (Rupasinghe & Yu, 2012). Processing may be achieved by using preservatives such as sugar, salt and vinegar, by drying, concentration or fermentation. Though production of alcoholic fermented beverages is mainly done by the fermentation of grape juice, it is also produced extensively from fruits other than grapes across the globe (Jarvis, 2001; Kumar *et al*., 2009; Isitua & Ibeh, 2010). During fermentation, yeast converts one mole of sugar into two moles of ethanol and two moles of $CO_2$ via glycolysis (Embden-Meyerhof pathway). A significant portion of sugar is used for the formation of biomass and other by-products (e.g. glycerol, organic acids, esters and higher alcohols), thereby reducing efficiency of the conversion of sugars into ethanol, which reaches 92 – 93%. Hence, using plums to develop fermented alcoholic beverages is a viable proposition in terms of value-addition and therefore, increased utilization of this crop.

To a great extent alcoholic fermented beverage quality is, related to overall aroma and therefore to the volatile compounds responsible for these aromas which produce a sensorial effect (Sáenz-Navajas *et al*., 2010). To fully be aware of chemical compounds within alcoholic fermented beverages that show sensory characteristics is important to obtain some information regarding both volatile composition and sensory properties (Capone *et al*., 2013). Gas chromatography is one of the important techniques of analysis for volatile components which contribute to the aroma of alcoholic fermented beverages. Equally important is the formation and the detection of methanol by using gas chromatography. Methanol is considered to be highly toxic whereby the ingestion or inhalation can cause blindness or death (Blinder *et al*., 1988). Methanol in alcoholic fermented beverages is formed from the demethoxylation of esterified methoxyl groups of the pectin polymer. Pectins which are present in fruits are composed of the methyl ester of alpha-1.4-linked, D-galacturonopyrasose units and is the general term for pectic substances which form the characteristic sugar-acid gels. Thus, the use of pectolytic enzymes is of major

importance to this study since it removes methoxyl groups from methylated pectic substances (pectin).

However, even more important than instrumental assays, sensory analysis initiates the detection and description of qualitative and quantitative sensory components of a product by a trained panel of judges (Meilgaard *et al*., 1999). Quantitative descriptive analysis is an informative tool and technique often used to provide complete sensory descriptions of a product such as alcoholic fermented beverages (Murria *et al*., 2001). Results obtained from descriptive analysis enable the relation of specific ingredients or process variables to specific changes in sensory attributes of food products in general including the alcoholic fermented beverages that are the focus of this study. From a product development perspective, descriptive data is essential in directing efforts on those product variables that are identified as different among relative to a target and to form fundamental interactions (Stone & Sidel, 2003). In terms of sensorial evaluation, multivariate analysis has been used for descriptive sensory evaluations. Principal component analysis (PCA) is frequently used as the statistical tool of analysis and has been applied to sensory results (Noble & Ebeler, 2002).

The aim of this study was to measure methanol, ethanol, titratable acidity, objective colour, total soluble solids and sensory profile as a function of yeast strain and percentage pulp in order to adapt current wine fermentation technologies to produce plum fermented beverage products using plums, an under-utilized agricultural produce.

## 3.3 Materials and methods

### *3.3.1 Preparation of the alcoholic fermented plum beverages*

#### *3.3.1.1 Fruit preparation*

Songold plums were obtained from Sandrivier and African pride plums were obtained from Mr Chris Smith (Agricultural Research Council, Infruitec-Nietvoorbij, Stellenbosch, South Africa). Only mature fruit was included when selecting plums for use in this study. Plums received from the supplier were placed into plastic fruit crates (20 kg) and placed into cold storage at (4˚C) to control the ripening for a maximum of two weeks. After storage, plums that were free from mould were thoroughly washed before cutting. Plums were cut in half by hand using stainless

steel paring knives, after which the stones were removed to prepare the fruit for the pulping process. Pulping was achieved using a fruit-pulper fitted with a 2 mm stainless steel sieve (Jas Enterprises, Rakhial Ahmedabad, India). During the pulping process, the plum halves were fed slowly through the fruit pulper to prevent blockage and it also allowed peels to be separated from the pulp. Pasteurization of the pulp was performed to ensure preservation of the pulp. This operation was carried out by using a tube-in-tube heat exchanger at a temperature of 92°C for 10 – 60 s, which was followed by hot-filling the pulp into 250 mL foil-laminate juice pouches, followed by heat sealing. The pouches were then placed in frozen storage at -15°C, thereby ensuring stability and consistent quality throughout the study.

*3.3.1.2 Product development*

Preliminary trials were conducted to establish formulations. A range of formulations were developed by combining different ratios of pulp at 17.5°B and added sugar as summarised in Tables 3.1 and 3.2.

The resulting formulations, namely Formulations 1 (Table 3.1) and 2 (Table 3.2) were prepared, inoculated, fermented, clarified and bottled. The resultant alcoholic fermented plum beverages were evaluated by wine researchers at the Post-harvest and Wine Technology division (Agricultural Research Council, Nietvoobij, Stellenbosch, South Africa).

The tasting sessions were conducted such that each sample (i.e. treatment combination) was evaluated twice, but on two separate days. Eighteen samples per session were presented to each panellist in ISO standard wine tasting glasses which were placed on a tray labelled with the relevant information. All samples were clearly marked with the percentage pulp, added sugar and yeast strain that were used. Approximately 100 mL per glass was served throughout.

Seven male judges, ranging in age from 25 to 65 participated in these taste sessions. These judges were all trained wine tasters and had extensive knowledge and experience in wine research and wine tasting.

After the two tasting sessions for each pulp-sugar-yeast combination, a general discussion took place, at the end of which the expert judges reached consensus on which formulation would produce the most sensorially acceptable beverage, based on flavour, i.e. aroma and taste. The best formulation was found to be Formulation 1 with the pulp percentage between 40 – 60% (Table 3.1). The selection was also

**Table 3.1** Alcoholic fermented plum beverage Trial 1 (Formulation 1)

| **Percentage pulp** | **40% ($m.m^{-1}$)** | | | **50% ($m.m^{-1}$)** | | | **60% ($m.m^{-1}$)** | | |
|---|---|---|---|---|---|---|---|---|---|
| Potential EtOH ($v.v^{-1}$) | **10** | 8 | 6 | **10** | 8 | 6 | **10** | 8 | 6 |
| Pulp (17.5°B) added (kg) | **1.80** | 1.80 | 1.80 | **2.25** | 2.25 | 2.25 | **2.70** | 2.70 | 2.70 |
| Sugar added (kg) | **0.63** | 0.44 | 0.25 | **0.55** | 0.36 | 0.17 | **0.47** | 0.28 | 0.09 |
| Water added (kg) | **2.07** | 2.26 | 2.45 | **1.70** | 1.89 | 2.08 | **1.33** | 1.52 | 1.71 |
| Total mass in (kg) | **4.50** | 4.50 | 4.50 | **4.50** | 4.50 | 4.50 | **4.50** | 4.50 | 4.50 |

**Table 3.2** Alcoholic fermented plum beverage Trial 2 (Formulation 2)

| **Percentage pulp** | **45% ($m.m^{-1}$)** | | | **50% ($m.m^{-1}$)** | | | **55% ($m.m^{-1}$)** | | |
|---|---|---|---|---|---|---|---|---|---|
| Potential EtOH ($v.v^{-1}$) | 10 | 8 | 6 | 10 | 8 | 6 | 10 | 8 | 6 |
| Pulp (17.5°B) added (kg) | 2.03 | 2.03 | 2.03 | 2.25 | 2.25 | 2.25 | 2.48 | 2.48 | 2.48 |
| Sugar added (kg) | 0.59 | 0.40 | 0.21 | 0.55 | 0.36 | 0.17 | 0.51 | 0.32 | 0.13 |
| Water added (kg) | 1.88 | 2.07 | 2.26 | 1.70 | 1.89 | 2.08 | 1.51 | 1.70 | 1.89 |
| Total mass in (kg) | 4.50 | 4.50 | 4.50 | 4.50 | 4.50 | 4.50 | 4.50 | 4.50 | 4.50 |

based on 10% ethanol (EtOH) measured in these beverage samples, this being the typical average EtOH content in white wine (Tabilo-Munizaga *et al*., 2014).

### *3.3.2 Production of alcoholic fermented plum beverages*

The selection of yeast strains used in the study was based on them being the most widely used commercially in the South African wine industry, as well as their capacity to enhance flavour or aroma through their ability to produce esters (Swiegers & Pretorius, 2005). The two yeast strains that were selected and used in the study were namely *Saccharomyces cerevisiae* (hybrid) VIN13 (ester forming) (Anchor Yeast, Cape Town, South Africa) and *Saccharomyces bayanus* N96 (N = Nuy wine cellar) (Anchor Yeast, Cape Town, South Africa) used in the fermentation of the must. Sugar (sucrose) was also used in the formulation and was obtained from a local supermarket. Wine was fermented in “Oom Tas” bottles with a capacity of 5 L, equipped with fermentation traps (Wine Machinery, Stellenbosch, South Africa). Kieselguhr, a commercial filtering aid, coarse pre-filter pads (Fibrafix AF 30, Filtrox, St. Gallen, Switzerland) and 4.5 µm fine filter pads (Filtrox, St. Gallen, Switzerland) were used in the filtration process. The samples of alcoholic fermented plum beverages produced were filled into 275 mL clear bottles and hermetically sealed with crown cork closures after filtering.

Fermentations were carried out at a temperature of 15$^{\circ}$C in a wine cellar at the Agricultural Research Council, Nietvoorbij, Stellenbosch, South Africa). The formulation (plum pulp, sugar and water) selected for the main study described in (section 3.3.1.2) was inoculated with 1.5 g of either VIN13 or N96 yeast strains according to the experimental design described in (section 3.3.5). Thereafter, pectolytic enzymes were prepared and 1.2 mL of each preparation was added to the pulp to increase the yield (Pectinex Ultra Mash, Novozymes, Bagsvaerd, Denmark) and to assist with clarification (Pectinex Ultra Clear, Novozymes, Bagsvaerd, Denmark). The latter enhances sedimentation of the must. Fermentations were performed in 52 fermentation vessels (“Oom Tas” bottles), each fitted with a fermentation trap (Wine Machinery, Stellenbosch, South Africa). The fermentation traps were inspected on a weekly basis to observe fermentation activity in the form of visible bubbles caused by $CO_2$ released during the fermentation. After five weeks, the traps were monitored daily for three weeks during which time the fermentation traps showed no further acitivity which indicated that the evolution of $CO_2$ had ceased and that the fermentation process was complete.

Since the fermentation vessels were not disturbed or physically displaced during fermentation, at the end of the fermentation, the lees were completely settled, obviating a further standing period. The clear fermented beverage in each “Oom Tas” bottle was racked from the lees at the cellar (at a temperature of 15°C). The beverage samples were then transferred into 10 L stainless steel vessels equipped with pressure inlet and outlet valves. Before the filtration procedure commenced, 50 g of Kieselguhr was added to each stainless steel vessel. Using pressure filter assemblies equipped with a coarse pre-filter (Fibrafix AF 30, Filtrox, St. Gallen, Switzerland), followed by a 4.5 µm filter pad (Filtrox, St. Gallen, Switzerland) and nitrogen gas at 200 kPa, the samples were filtered and bottled. Each bottle was capped with a crown closure and each batch yielded 12 bottles (275 mL) of alcoholic fermented plum beverage. After bottling, the bottled beverage samples were subjected to pasteurization in a dehydrator at an air temperature of 80°C for 45 minutes, followed by cooling in water at 10°C.

### *3.3.3 Chemical analyses*

#### *3.3.3.1 Materials*

Unless otherwise specified, all the chemicals used in this study were of Analar grade and chemical reagents were prepared according to standard analytical procedures.

#### 3.3.3.2 *Linearity curve*

Ethanol (EtOH) and methanol (MeOH) (Merck, Darmstadt, Germany) used in GC analysis was of chromatography grade. Milli-Q water (Millipore, Bedford, MA, USA) (18.2 $M\Omega.cm^{-1}$) was used for dilutions of standards. A linearity curve was constructed using standard solutions at 0.5, 1, 2, 3 and 4% ($v.v^{-1}$) for MeOH (Figure 3.1) and standard solutions at 5, 10, 15, 20 and 25% ($v.v^{-1}$) for EtOH (Figure 3.2). Five standards per concentration (n = 5) were analysed and the multiple correlation coefficient ($R^2$) and regression coefficient (R) were used to determine whether the peak areas plotted would be linear over the concentration range. The linearity curve was also assessed to ensure that the method was sufficiently sensitive over the concentration range of MeOH and EtOH levels anticipated to be present in the alcoholic fermented plum beverage samples. The LOD and LOQ limits were calculated based on the standard deviation (SD) of the response and the slope (S) of the calibration curve at levels approximating the LOD according to the formula:

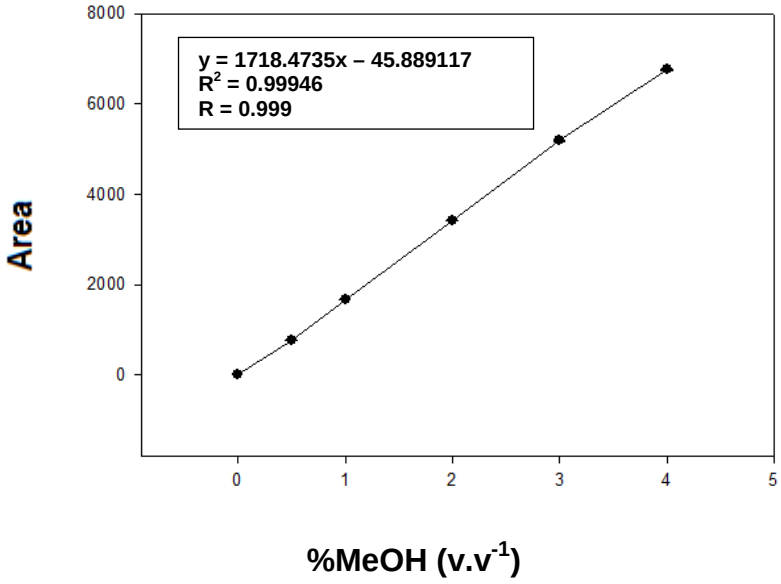


**Figure 3.1** Graph depicting the linear trend with respect to area recorded when a series of **MeOH** standard solutions of increasing concentration were analysed (n=5)

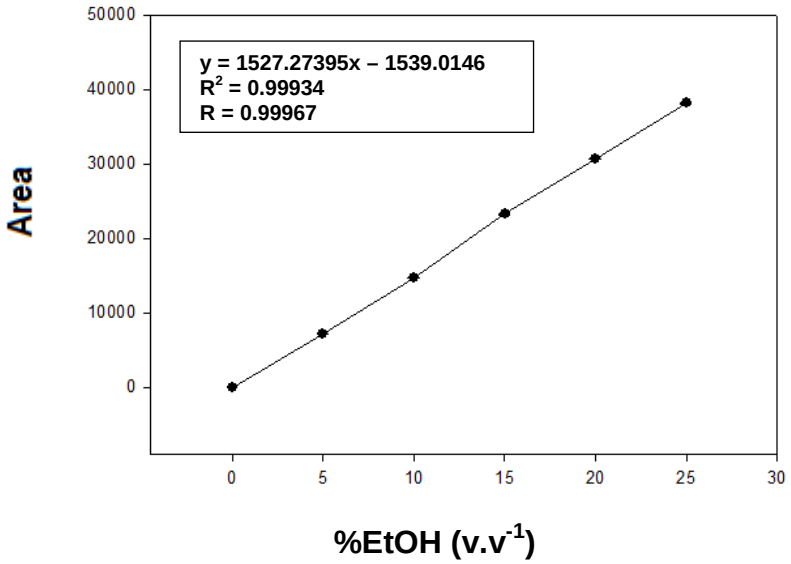


**Figure 3.2** Graph depicting the linear trend with respect to area recorded when a series of **EtOH** standard solutions of increasing concentration were analysed (n=5)

LOD = 3(SD/S). The LOQ was determined using the response SD and the slope of the calibration curve according to the formula: LOQ = 10(SD/S). The calibration curve was created using SigmaPlot® 2001 for Windows® (Version 6:10, SPSS Inc., Chicago, IL, USA).

#### *3.3.3.3 Repeatability*

The repeatability or relative precision of the method was established by measuring replicates of standard solutions of MeOH and EtOH of known concentration over two consecutive sessions on the same day and over two consecutive days, i.e. intra-day and inter-day assays. Five replicates of the MeOH and EtOH standards were analysed in two sessions on one day and the intermediate precision was determined by analysing two sets of 10 replicates of MeOH and EtOH standards on two consecutive days.

#### *3.3.3.4 Gas chromatography assay*

The MeOH and EtOH in the alcoholic fermented plum beverages were analyzed separately, but directly, i.e. without any extraction process. One mL aliquots of each of the samples were pipetted into two mL screw-cap clear glass vials with septa (Chemetrix, South Africa). An Agilent 7890 A GC system equipped with a split/splitless injector and a flame ionisation detector was used (Agilent Technologies, Waldbron, Germany). The analysis for MeOH was performed with an – HP 88 column (100 m × 0.25 mm internal diameter, 0.2 µm film thickness; J&W Scientific, Folsom, California, USA). The analysis for EtOH was performed with a DB23 column (60 m × 0.25 mm internal diameter, 0.15 µm film thickness; J&W Scientific, Folsom, California, USA). The GC parameters for both MeOH and EtOH were set as follows. The temperature of the injector and detector was set at 250°C and 300°C, respectively. The oven temperature was set at 150°C and the samples analyzed isothermically for 6 min. Nitrogen (Air Liquide, Paris, France) was used as the carrier gas at 22.3 kPa, with a split vent of 40 $mL.min^{-1}$. Injections of 1 µL were made in split-mode with a speed ratio of 50:1. The EtOH and MeOH (Merck, Germany) in the samples were identified by comparing the retention times of the samples with those of 99.9% HPLC grade MeOH and EtOH standard solutions. Quantification of MeOH and EtOH was performed using Chemstation software (version B.04.01) (Agilent Technologies, Waldron, Germany) after determining the detector response factor for both MeOH and EtOH in each sample. The quantification of the MeOH and EtOH was achieved using the external standard method.

*3.3.3.5 Titratable acidity*

The titratable acidity (TA) of the alcoholic fermented plum beverage was determined by titrating a 5 mL aliquot of alcoholic fermented plum beverage with 0.1 N NaOH to a pH endpoint of 8.2 using an automated titrator (Crison compact titrator, version D, Alella, Spain). The TA of the samples was measured in grams malic acid per litre.

3.3.3.6 *Total soluble solids analysis*

The total soluble solids (TSS) content of the plum pulp and alcoholic fermented fruit beverage samples were measured in °Brix using an Atago Palette PR-101 refractometer (Tokyo, Japan).

3.3.3.7 *Spectrophotometric measurements*

Alcoholic fermented plum beverage samples were placed in 2 mL sample cuvettes. Spectrophotometric measurements for colour of the alcoholic fermented beverage samples were performed using a colorimeter (Model CM – 5, Konica Minolta Sensing Inc., Osaka, Japan). Each measurement was based on the CIELab colour co-ordinates, namely $L^*$, $a^*$, $b^*$, C and h. Colour values were expressed as $L^*$ (whiteness or brightness/darkness), $a^*$ (redness/greenness), $b^*$ (yellowness/blueness), C (Chroma) expresses the degree of colour for an area viewed on CIELab colour coordinates viewed in relation to its brightness, which is calculated as $(a^* + b^*)^{½}$ and h (hue) angle is derived from the two coordinates a* and b* and is determined as arctan b*/a*, hue angle is expressed on a 360° grid where 0° = bluish-red, 90° = yellow, 180° = green, 270° = blue and 360° = red (Sahin & Sammu, 2006).

### *3.3.4 Sensory evaluation*

*3.3.4.1 Sensory panel composition*

Nine female judges and one male, ranging in age from 25 to 65 participated in the study. They were selected based on availability and product interest. Most of them had extensive experience with descriptive analysis of a wide range of products.

*3.3.4.2 Panel Training*

The training of the panel was conducted according to the consensus method described by Lawless and Heyman (1998). The panellists were informed about the background and objectives of the study and instructed on the sensory evaluation procedure. They were

instructed to remove the plastic cap from the serving glass, swirl the glass three times in an anti-clockwise rotation and then evaluate the aroma of the alcoholic fermented plum beverage sample. Thereafter, they were instructed to evaluate the flavour, taste and mouthfeel by sipping a mouthful of the beverage. The panel was also instructed to cleanse their palate in-between samples using water and unflavoured water biscuits.

During the first part of the training, panellists were exposed to a number of reference standard samples (Table 3.3), to familiarise themselves with the product and the analysis protocol. Thereafter, panellists were given alcoholic fermented plum beverage samples, where the panellists were then instructed to compare the aroma attributes of the reference standards to the aroma of the samples. Flavour, taste, aroma and mouthfeel terminology, also known as descriptive terms (or descriptors) were suggested and deliberated by the panel members and each new term was recorded. Aroma was defined as the fragrance or odour perceived through orthonasal analysis, while flavour referred to the retronasal perception in the mouth. The term ‘taste’ was used to describe the basic taste modalities, i.e. sweet, sour, salty and bitter. Mouthfeel was described as the tactile sensation that occurred in the oral cavity after sipping the alcoholic fermented plum beverage (Gawel *et al*., 2000). Relationships and redundancies among the terms were discussed and definitions and actual reference standards for the prevailing sensory descriptors were obtained (Table 3.3).

During 24 one-hour sessions, the alcoholic fermented plum beverage samples were analyzed and compared to one another by the panel based on the descriptors. During these training sessions twelve aroma and six flavour, taste and mouthfeel descriptors were generated for the beverage samples. Ten of these terms were selected for inclusion in the sensory analysis based on their frequency of being mentioned by the panel during the training phase. The selected descriptors, i.e. sensory profiling attribute terms included four aroma descriptors, one flavour descriptor, four taste descriptors and one mouthfeel descriptor (Table 3.4). A score sheet was then developed which was used by the panel to score the intensity of each of the 10 descriptors on a 100 mm uninstructed line scale anchored on both sides with two word descriptors – “Absent” and “Prominent”. Figure 3.3 depicts a representation of the evaluation form (the lines are not to scale). During the final training sessions, the panel practised intensity ratings of individual attributes on the line scales using the standards depicting intensity extremes for all of the descriptors. Maximum and minimum intensity values for the ten attributes were discussed and compared to the attribute intensity scores that had been awarded by the panel.

**Table 3.3** Aroma attributes and reference standards presented to the sensory panel during panel training sessions

| Aroma attributes | Physical standards supplied to the panel |
|---|---|
| **Fruity** | |
| Apple | Fresh apple (2 slices) |
| Plum | Plum (Sensient 1003899) 10 µL in 100 mL water |
| Cherry | Cherry (Sensient 1005440) 20 µL in 100 mL water |
| **Berry-like** | |
| Mixed berry | Berry blend (Sensient F17921) 10 µL in 100 mL water |
| Raspberry | Natural Raspberry (Sensient 1012887) 20 µL in 100 mL water |
| Strawberry | Strawberry key 2 (Sensient 1100851) 10 µL in 100 mL water |
| **Woody** | |
| Planky | 2 g of plank shavings in 100 mL water |
| **Whisky-like** | |
| Whisky 1 | Whisky (Three Ships) 2 mL in 30 mL water |
| Whisky 2 | Whisky (First Watch) 2 mL in 30 mL water |
| Whisky 3 | Whisky (Three Ships 5 year) 2 mL in 50 mL water |

**Table 3.4** Aroma, flavour (F), taste (T) and mouthfeel (MF) sensory attributes selected by the panel for descriptive analysis

| **Aroma attributes** | **Flavour, taste and mouthfeel attributes** |
|---|---|
| Fruity | Fruity (F) |
| Sweet-associated[1] | Sweet (T) |
| Woody | Sour (T) |
| Yeasty | Bitter (T) |
| | Astringent (MF) |
| | Lingering Aftertaste (T) |

[1]Sweet-associated fruity aroma, resembling fresh fruit.

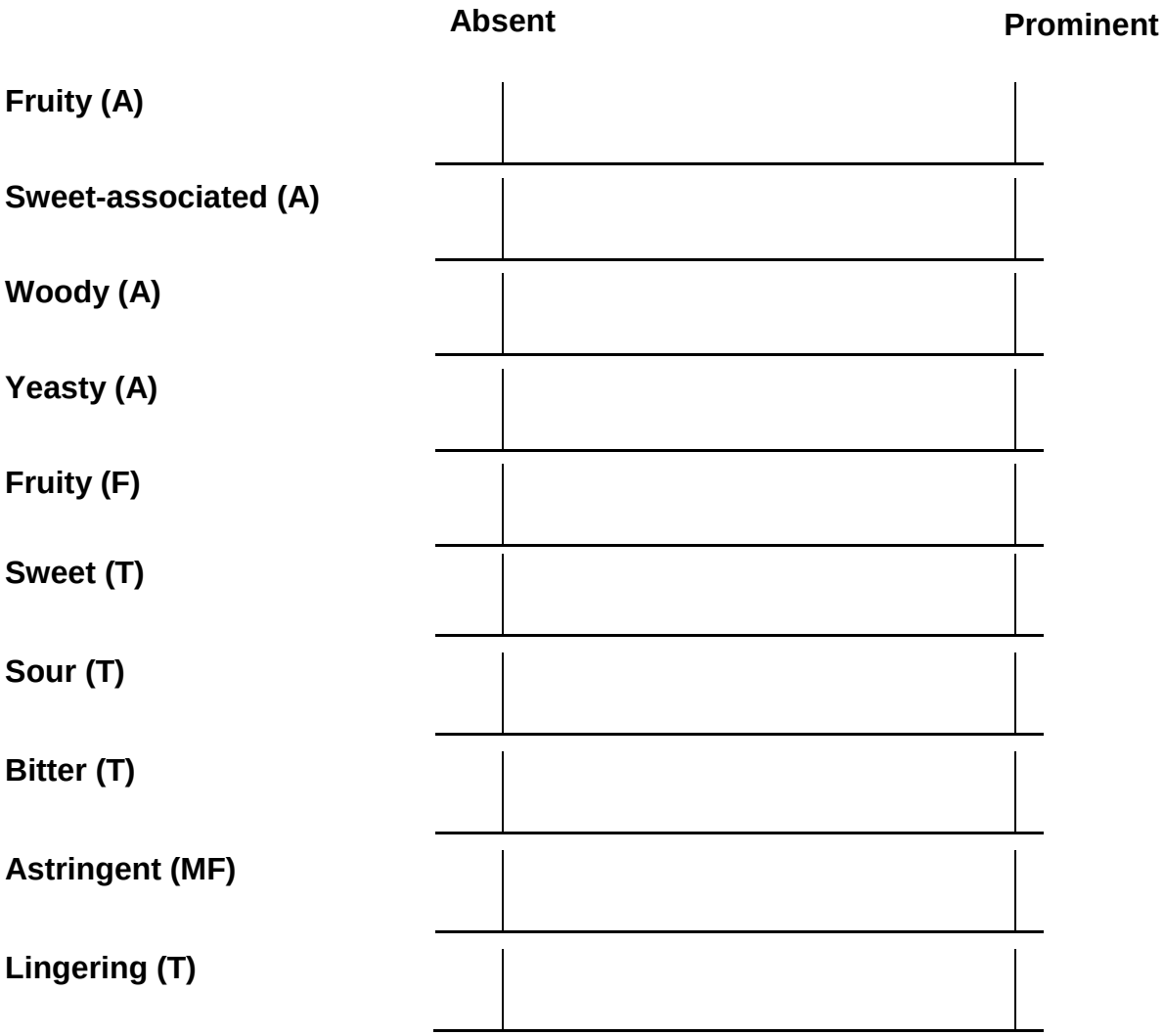


**Figure 3.3** Representation of the uninstructed line scale with the ten attributes for intensity rating used during the descriptive analysis. The length of the scale on the evaluation form was 100 mm

#### 3.3.4.3 *Samples and sample serving*

Bottled alcoholic fermented plum beverage samples were used for the descriptive sensory analysis of the beverage. Samples were presented to the panel in ISO standard wine tasting glasses, placed on a traysheet labelled with relevant information regarding the samples in question that represented the experimental design described in (section 3.3.5). Samples were labelled with random three-digit codes and presented to each panellist. Approximately 30 mL per glass was served, each covered with a plastic cap to prevent evaporation and loss of volatiles.

#### 3.3.4.4 *Intensity rating*

The panel was requested to use the score cards to rate the intensities of the 10 attributes for each of the alcoholic fermented plum beverages during six sessions spread out over two weeks. One session was conducted per day and a maximum of 12 samples were analyzed per session. Panellists were requested to take a 10 min break after every 3 samples to avoid sensory fatigue. Each sample was analyzed in triplicate, on three non-consecutive days in order to test for panel reproducibility and reliability.

### ***3.4 Experimental design***

The physicochemical experimental design was a 3 x 2 design with three pulp concentrations and two yeast strains VIN13 and N96. The response variables were titratable acidity, $^{\circ}$Brix, pH, ethanol (EtOH), methanol (MeOH) and colour ($L^*$, $a^*$, $b^*$, C, h). The sensory evaluation experimental design comprised of 3 x 2 x 10 x 3 factors, namely three pulp concentrations, two yeast strains, ten panellists and three sensory evaluation sessions. The response variables were fruity aroma, sweet-associated aroma, woody aroma, yeasty aroma, sweet taste, sour taste, bitter taste, lingering aftertaste and fruity flavour.

### ***3.5 Data analysis***

The physicochemical data was subjected to a multivariate analysis of variance (ANOVA) to ascertain whether the main effects resulted in significant differences in response variables. The Duncan's multiple comparison post hoc test was used to test significant differences ($p < 0.05$) between individual means. The sensory evaluation data was subjected to factor analysis (Principle component analysis (PCA)) and multivariate ANOVA, IBM® SPSS® statistical software (Version 22; IBM Corporation, New York, USA) was used. Microsoft®

Excel 2010 software (Maryland, USA) was used to construe a spider plot as a graphic summary of the data.

## 3.6 Results and Discussion

### *3.6.1 Linearity*

An acceptable linearity was demonstrated between the specific MeOH and EtOH peak areas and concentrations of the injected standards over a range of concentrations, between 0.5 – 4% ($v.v^{-1}$) for MeOH and 5 – 25% ($v.v^{-1}$) for EtOH, respectively (Table 3.5). The regression coefficient (R) for MeOH was 0.99972 and correlation coefficient ($R^2$) = 0.99946 (Figure 3.1) and the EtOH regression coefficient (R) was 0.99967 and the correlation coefficient ($R^2$) = 0.99934 (Figure 3.2). This clearly indicated that the linearity was satisfactory for MeOH and EtOH. The LOD of MeOH was 0.00000142 $\mu g.mL^{-1}$ and the LOQ was 0.00000473 $\mu g.mL^{-1}$ (Table 3.5). The LOD of EtOH was 0.000603 $\mu g.mL^{-1}$ and the LOQ was 0.00201$\mu g.mL^{-1}$ (Table 3.5). The calibration procedure was performed according to the AOAC guidelines (Anon., 2002), and the results confirmed that the concentration range of interest over five points having equal spacing was a suitable calibration pattern, while a high correlation co-efficient of > 0.99 is proof of a good quality linear fit. A similar study done on alcoholic fermented beverages by Fariña *et al*. (2007) also showed a correlation coefficient of > 0.99. Based on this result the methods used to determine R and $R^2$ for MeOH and EtOH was validated using the calibration procedure, confirming that the method was reliable.

### *3.6.2 Precision*

The analytical precision was summarised in Table 3.6 for both MeOH and EtOH. Repeatability precision was determined by analysing aliquots of the same sample numerous times. This includes simultaneous and consecutive replicates of the sample (Van Wyk & Britz, 2012). Five replicates of the MeOH and EtOH standards were analysed in two consecutive sessions on one day (simultaneous replicates). The intermediate precision was determined by analysing 10 sample replicates of the MeOH and EtOH standards on two consecutive days. The mean ± standard deviation (SD) was calculated and the probability was non-significant ($p > 0.05$) (Table 3.6) in all cases, indicating acceptable precision of the analytical methods.

**Table 3.5** The limits of detection (LOD), quantification (LOQ) and linear range of methanol (MeOH) and ethanol (EtOH)

| Parameter | MeOH | EtOH |
|---|---|---|
| **Linear range** | 0.5% – 4% ($v.v^{-1}$) | 5% – 25% ($v.v^{-1}$) |
| **LOD** | 0.0000014 ($\mu g.mL^{-1}$) | 0.00060 ($\mu g.mL^{-1}$) |
| **LOQ** | 0.0000047 ($\mu g.mL^{-1}$) | 0.0020 ($\mu g.mL^{-1}$) |

**Table 3.6** Method precision based on repetitive analyses of MeOH and EtOH standards, assayed on two consecutive days

| Sample | %MeOH ($v.v^{-1}$) (Mean ± standard deviation) | %EtOH ($v.v^{-1}$) (Mean ± standard deviation) | p-value[1] |
|---|---|---|---|
| **Intra-day** | | | |
| Morning (n=5) | 2.09 ± 2.05 | 15.03 ± 62.72 | |
| | | | p > 0.05 |
| Afternoon (n=5) | 2.02 ± 2.06 | 14.91 ± 62.31 | |
| **Inter-day** | | | |
| Day 1 (n=10) | 2.09 ± 1.83 | 14.97 ± 55.57 | |
| | | | p > 0.05 |
| Day 2 (n=10) | 2.12 ± 1.97 | 14.99 ± 54.97 | |

[1]Student's t-tests (unpaired, two-tailed) were performed to establish whether the intra-day and inter-day results differed significantly, $p < 0.05$ indicates significance.

### *3.6.3 Physicochemical analysis*

It is clear from the data (Table 3.7) that the titratable acidity (TA) expressed as grams malic acid per litre ranged between 7.64 – 12.95, increasing as the pulp concentration increased from 40% ($m.m^{-1}$) to 60% ($m.m^{-1}$) pulp concentration. The values for the formulation containing 60% pulp (both yeast strains) differed significantly ($p < 0.05$) from the formulations containing 40% and 50% pulp which were lower. This is the result of incorporating more pulp in the formulation with a simultaneous increase in acidity originating from the pulp. These results were congruent with those reported by Joshi *et al*. (2012) in a similar study on alcoholic fermented plum beverages.

The total soluble solids (TSS), measured in °Brix, ranged from 8.30 to 8.95 (Table 3.7). Even though there were no significant differences ($p > 0.05$) among treatments (yeast or %pulp), the results obtained were similar to the results reported by Joshi *et al*. (2009) who also developed alcoholic fermented beverages from plums, with the TSS ranging from 7.2 – 7.6.

The pH values ranged between 3.45 – 3.55 with differences not significant ($p > 0.05$) among treatments (Table 3.7). Hence, the significantly higher TA observed with 60% pulp did not translate into significantly lower pH values. The pH range observed in this study is comparable to that of white wines produced in South Africa where the pH ranges between 3.11 – 3.84 (Nieuwoudt *et al*., 2002), and which is favourable for storage stability, since this relatively low pH deters spoilage (Jackson, 2008).

The %EtOH ($v.v^{-1}$) ranged between 11.60 – 11.99% (Table 3.7), which was slightly higher than the target %EtOH based on the beverage formulations, namely 10% ($v.v^{-1}$). However, the %EtOH ($v.v^{-1}$) range measured in this study can be compared to the typical range of South African styled white wine such as Sauvignon blanc where the %EtOH ($v.v^{-1}$) range is between 11.8 – 11.9 %EtOH ($v.v^{-1}$) (King *et al*., 2010). Differences in %EtOH among treatments were not significant ($p > 0.05$).

MeOH was not detected in the present study and therefore could not be quantified (Table 3.7). This result is important because the production of MeOH in alcoholic fermented beverages is not only considered an undesirable component in the final product, but is identified to be toxic to humans when consumed even in relatively low concentrations (Campos *et al*., 2010). The results of this study compare favourably with other studies. For example, the methanol content in South African young white wines were reported in a study by Louw *et al*. (2010) to range between 25 – 83 $mg.L^{-1}$.

**Table 3.7** Physicochemical profile of alcoholic fermented plum beverage samples[1]

| Treatment | | Chemical profile | | | | | Physical profile | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| %Pulp | Yeast | Titrable acidity (TA) as malic acid ($g.L^{-1}$) | Brix (°B) | pH | EtOH ($v.v^{-1}$) | MeOH ($v.v^{-1}$) | L* | a* | b* | C | h |
| 40 | VIN 13 | 7.64 ± 2.72[a] | 8.45 ± 0.56 | 3.45 ± 0.10 | 11.60 ± 0.46 | n.d[2] | 94.85 ± 3.42 | 4.10 ± 2.91 | 17.20 ± 8.44 | 16.55 ± 8.54 | 85.12 ± 7.50 |
| | N 96 | 8.63 ± 2.09[a] | 8.30 ± 0.68 | 3.52 ± 0.05 | 11.82 ± 0.40 | n.d | 94.34 ± 4.72 | 3.04 ± 3.68 | 14.48 ± 9.75 | 13.87 ± 10.31 | 87.94 ± 11.58 |
| 50 | VIN 13 | 8.28 ± 1.58[a] | 8.95 ± 0.40 | 3.52 ± 0.07 | 11.84 ± 0.56 | n.d | 94.57 ± 2.46 | 3.33 ± 2.47 | 16.82 ± 9.78 | 14.07 ± 8.23 | 85.33 ± 4.22 |
| | N 96 | 9.81 ± 2.86[a] | 8.36 ± 0.36 | 3.50 ± 0.02 | 11.62 ± 0.49 | n.d | 93.59 ± 2.86 | 2.22 ± 2.11 | 10.74 ± 7.34 | 10.13 ± 4.68 | 84.88 ± 3.56 |
| 60 | VIN 13 | 12.95 ± 0.19[b] | 8.43 ± 0.50 | 3.55 ± 0.11 | 11.64 ± 0.42 | n.d | 94.76 ± 3.59 | 4.30 ± 2.25 | 16.89 ± 6.61 | 16.82 ± 8.11 | 86.57 ± 10.33 |
| | N 96 | 12.47 ± 2.40[b] | 8.65 ± 0.80 | 3.54 ± 0.10 | 11.99 ± 0.29 | n.d | 94.18 ± 2.92 | 3.01 ± 2.43 | 15.35 ± 7.19 | 15.60 ± 7.46 | 84.88 ± 6.41 |

[1]Results reported as mean ± standard deviation. A multivariate analysis of variance (ANOVA) with Duncan's multiple comparison post-hoc test was performed and [a – b] Means with different letter superscripts in each column are significantly different ($p \leq 0.05$). [2]n.d. = not detected.

The alcoholic fermented beverage samples were observed to be “yellow” in colour. The results (Table 3.7) confirmed that there were no significant differences ($p > 0.05$) between treatments in terms colour space coordinates L*, a*, b*, C and h. The ranges of all the colour coordinates in all treatments were L* = 94.18 – 94.85, a* = +2.22 – +4.30, b* = +10.74 – +17.20, C = 10.13 – 16.82 and h = 84.88 – 87.94. The CIELab colour coordinates typical of white wine measured during a twelve month shelf-life period by Recemales *et al*. (2006) were L* = 100.11, a* = -0.21, b* = +3.87, C = 5.15 and h = 106.03. The most notable differences between these and the measurements for the plum beverage in this study were low positive values for $a^*$ (redness) and low positive $b^*$ values (yellowness), while the white wines had low negative $a^*$ (green) and lower positive $b^*$ (yellow) values (Recemales *et al*., 2006), thus a more intense yellow. Therefore, when comparing the CIELab colour coordinates of alcoholic fermented plum beverages produced in this study to that of white wines it is clear that the two types of alcoholic beverages are similar in terms of colour. Hence, the processing parameters as described in section 3.3.2 resulted in alcoholic fermented plum beverages that were comparable to typical white wines in terms of all the aforementioned physicochemical parameters.

### *3.6.4 Sensory evaluation*

Principal component analysis (PCA) was performed to summarise the sensory data. When reviewing the PCA results, the correlation was not strong enough between dependent variables (DV), namely fruity aroma, sweet-associated aroma, woody aroma, yeasty aroma, sweet taste, sour taste, bitter taste, lingering aftertaste and fruity flavour. This means that the information inherent in each DV was unique, as it did not influence the response to any other. The Kaiser-Meyer-Olkin Measure of Sampling Adequacy (KMO) test result was 0.695, with the ideal value ≥ 0.8, but since it was significant ($p < 0.001$), the components with Eigen values ≥ 1.00 were extracted. The extraction yielded three components, with component 1 explaining only 26.53% of the total cumulative variance, component 2 only 43.70% and component 3 only 59.99% of the total cumulative variance. Hence, the three components explained less than 60% of the total cumulative variance, while the ideal is ≥ 80%. Moreover, this means that 40% of the information contained in the data would be lost when using this data reduction tool. Hence, PCA was not a suitable tool to explain variability in the judgement of the trained panellists concerning the response variables. Instead, a spider plot was used to summarise the data (Figure 3.4). The lines on the spider plot, describing the individual curves

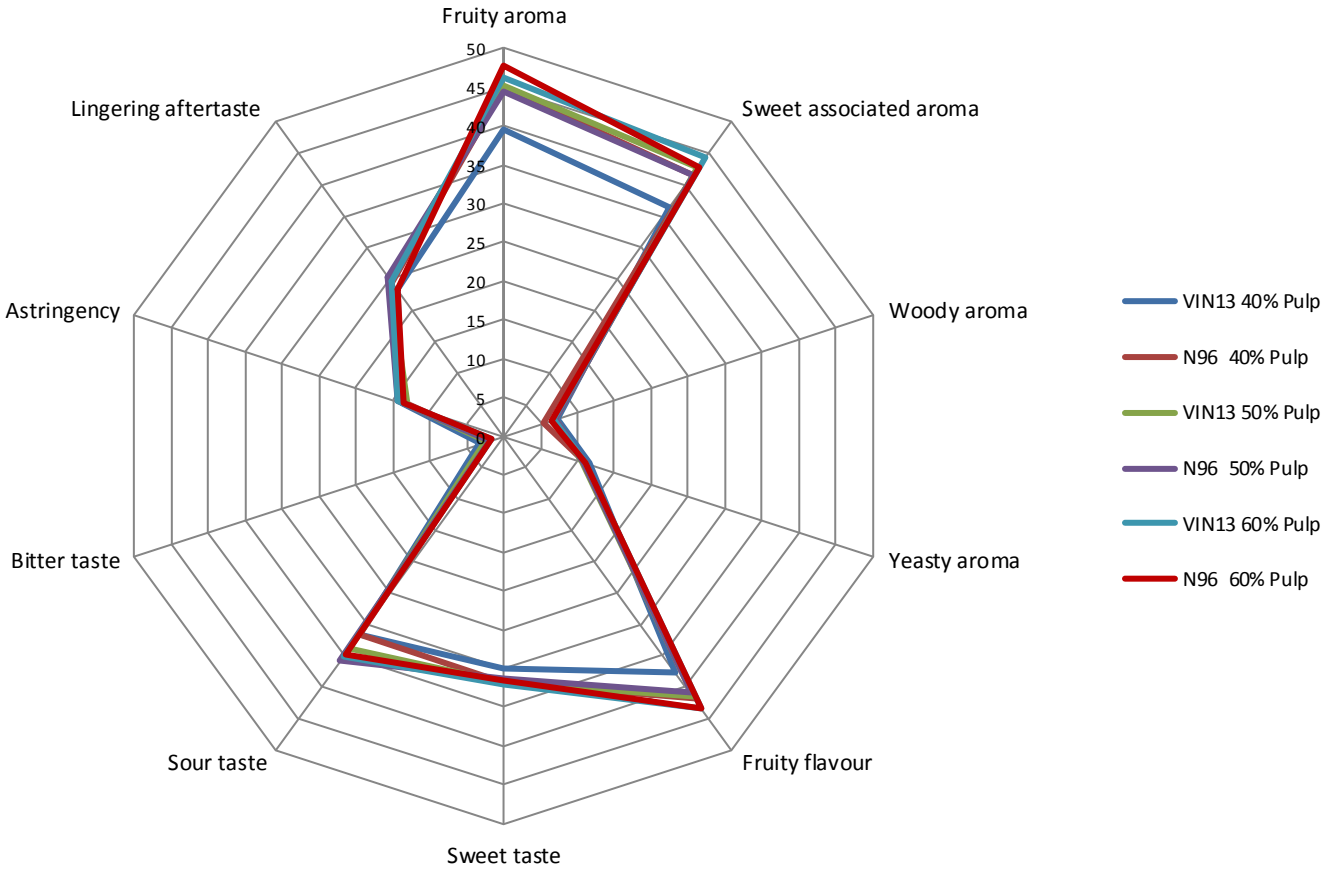


**Figure 3.4** Spider plot depicting the flavour profile of the six different alcoholic fermented plum beverage samples

corresponding to the different treatments, virtually coincide (Figure 3.4). This signifies that the treatments had a minimal effect on the responses of the panel.

In Table 3.8 the panel responses for woody aroma, yeasty aroma and lingering aftertaste were significantly different ($p < 0.05$) for sensory session 1 compared to sessions 2 and 3. Overall, woody aroma ranged between 4.62 – 8.72 and yeasty aroma ranged between 8.57 – 14.20. Hence, both aroma attributes were scored at the lower end of the line scale (Figure 3.3). The mean scores for lingering aftertaste ranged between 20.50 – 26.70, i.e. although double the highest score for yeasty aroma, it was still closer to “Absent” on the line scale.

Fruity aroma and sweet associated aroma were rated significantly higher as the pulp concentration increased from 40% to 50% ($p < 0.05$), but was not rated significantly different ($p > 0.05$) as the pulp concentration increased from 50% to 60%. However, the scores for fruity flavour at 60% pulp concentration were significantly different ($p < 0.05$) for all three sessions, with this pulp concentration resulting in the most intense fruity flavour. The result (Table 3.8) agrees with that observed on the spider plot (Figure 3.4) where overall 60% pulp concentration resulted in the highest fruity flavour.

The differences in sweet taste between samples were not significant ($p > 0.05$). Hence, the different treatments did not affect the panel’s response in terms of sweet taste. This agreed with the sugar levels that were measured in $^{\circ}$Brix which were all very similar and not significantly different ($p > 0.05$) (Table 3.7).

Sour taste was rated significantly lower ($p < 0.05$) for 40% pulp concentration, than for 50% and 60%, while the panel rated the sour taste most intense in most of the samples containing 50% pulp (Table 3.8). As far as TA is concerned, these results partially agreed with the results (Table 3.7), with the lowest TA recorded for 40% pulp. However, the highest TA was measured for 60% and not for 50% pulp (Table 3.7) while the pH for all pulp concentrations were similar (Table 3.7), the panels overall response for sourness ranged between 31.00 – 45.75, i.e. approximately midway between “Absent” and “Intense”. This anomaly could be explained by the fact that fruitiness was most intense at 60% pulp concentration thereby making the higher level of sourness more acceptable to the panel.

Bitter taste was rated significantly different ($p < 0.05$) for 40% pulp concentration samples. However, the results showed inconsistency in the sense that no clear trend was identifiable, since in some cases the bitterness in 50% pulp concentration samples were rated most prominent, while in other cases the 40% pulp concentration samples were rated most bitter. This could be attributed to the fact that phenols present in plum alcoholic

**Table 3.8** Quantitative descriptive analysis results[1] for alcoholic fermented plum beverages

| Treatments | | | Sensory attributes | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Session | Yeast | Pulp | Fruity aroma | Sweet-associated aroma | Woody aroma | Yeasty aroma | Sweet taste | Sour taste | Bitter taste | Astringency | Lingering aftertaste | Fruity flavour |
| 1 | VIN 13 | 40 | 41.60 ± 10.00$^{a}$ | 39.42 ± 10.75$^{a}$ | 8.22 ± 7.18 | 14.20 ± 6.70 | 29.62 ± 12.53 | 32.47 ± 11.97$^{a}$ | 4.92 ± 5.37$^{a}$ | 13.18 ± 5.20 | 20.50 ± 20.59 | 38.90 ± 10.96$^{a}$ |
| | | 50 | 47.07 ± 8.07$^{b}$ | 47.55 ± 8.78$^{b}$ | 8.72 ± 4.40 | 11.15 ± 6.25 | 33.40 ± 10.37 | 34.32 ± 11.23$^{b}$ | 1.50 ± 2.75$^{b}$ | 12.33 ± 6.23 | 25.85 ± 22.06 | 41.22 ± 10.15$^{b}$ |
| | | 60 | 47.50 ± 9.50$^{b}$ | 40.67 ± 10.40$^{b}$ | 7.82 ± 7.38 | 12.02 ± 5.63 | 29.57 ± 9.65 | 32.52 ± 9.77$^{b}$ | 1.50 ± 2.91$^{b}$ | 13.43 ± 4.58 | 22.27 ± 19.97 | 38.10 ± 11.77$^{a}$ |
| | N 96 | 40 | 42.87 ± 8.98$^{a}$ | 39.45 ± 9.44$^{a}$ | 6.90 ± 5.14 | 13.85 ± 5.74 | 32.20 ± 9.91 | 31.62 ± 12.78$^{a}$ | 1.92 ± 3.66$^{b}$ | 13.70 ± 5.78 | 24.72 ± 21.25 | 41.72 ± 9.90$^{a}$ |
| | | 50 | 46.25 ±11.77$^{b}$ | 47.62 ± 6.96$^{b}$ | 8.17 ± 6.76 | 10.00 ± 8.03 | 31.02 ± 11.27 | 36.87 ± 10.21$^{b}$ | 2.15 ± 3.49$^{a}$ | 15.15 ± 5.85 | 24.77 ± 22.24 | 40.87 ± 11.31$^{a}$ |
| | | 60 | 46.45 ± 9.61$^{b}$ | 42.07 ± 10.57$^{b}$ | 7.77 ± 5.09 | 11.22 ± 7.02 | 29.40 ± 7.77 | 34.65 ± 11.18$^{b}$ | 1.97 ± 2.68$^{b}$ | 13.92 ± 4.69 | 21.47 ± 20.65 | 39.00 ± 10.94$^{b}$ |
| 2 | VIN 13 | 40 | 41.12 ± 13.18$^{a}$ | 37.05 ± 10.63$^{a}$ | 5.50 ± 5.69 | 9.27 ± 6.20 | 30.45 ± 11.72 | 31.72 ± 10.78$^{a}$ | 2.20 ± 3.59$^{b}$ | 15.45 ± 4.69 | 25.25 ± 23.97 | 38.35 ± 10.50$^{a}$ |
| | | 50 | 44.80 ± 9.47$^{b}$ | 41.72 ± 9.29$^{b}$ | 6.75 ± 5.48 | 11.90 ± 4.85 | 29.30 ± 10.86 | 35.50 ± 10.65$^{b}$ | 2.37 ± 3.83$^{b}$ | 13.33 ± 5.17 | 23.67 ± 21.62 | 40.35 ± 8.12$^{a}$ |
| | | 60 | 47.12 ± 7.47$^{b}$ | 44.67 ± 10.83$^{b}$ | 5.32 ± 5.11 | 10.25 ± 5.13 | 31.70 ± 11.90 | 40.05 ± 8.74$^{b}$ | 1.62 ± 3.26$^{a}$ | 15.65 ± 4.66 | 25.05 ± 26.38 | 45.90 ± 11.47$^{b}$ |
| | N 96 | 40 | 45.57 ± 7.37$^{b}$ | 44.15 ± 10.09$^{a}$ | 4.87 ± 5.08 | 8.62 ± 7.82 | 32.35 ± 11.83 | 31.00 ± 10.72$^{a}$ | 1.82 ± 2.57$^{b}$ | 12.37 ± 4.51 | 23.90 ± 22.26 | 43.12 ± 10.56$^{a}$ |
| | | 50 | 42.05 ± 11.80$^{a}$ | 41.72 ± 9.29$^{b}$ | 6.75 ± 4.41 | 11.90 ± 4.85 | 43.50 ± 4.94 | 45.75 ± 2.47$^{b}$ | 2.07 ± 3.40$^{a}$ | 14.32 ± 4.51 | 25.75 ± 22.69 | 42.25 ± 11.63$^{a}$ |
| | | 60 | 47.42 ± 9.46$^{b}$ | 43.80 ± 9.02$^{b}$ | 6.50 ± 4.90 | 12.97 ± 8.82 | 12.17 ± 12.89 | 36.62 ± 7.27$^{b}$ | 1.40 ± 2.90$^{b}$ | 13.00 ± 5.38 | 23.62 ± 22.79 | 43.55 ± 8.86$^{b}$ |
| 3 | VIN 13 | 40 | 36.10 ± 15.29$^{a}$ | 32.80 ± 14.03$^{a}$ | 7.80 ± 4.93 | 11.25 ± 7.50 | 29.72 ± 12.07 | 30.42 ± 10.47$^{a}$ | 2.75 ± 4.29$^{b}$ | 13.73 ± 5.36 | 24.60 ± 25.20 | 36.02 ± 12.14$^{b}$ |
| | | 50 | 43.60 ± 10.15$^{b}$ | 38.67 ± 10.42$^{b}$ | 5.45 ± 4.77 | 8.57 ± 5.65 | 32.52 ± 10.59 | 31.57 ± 8.56$^{b}$ | 3.25 ± 4.18$^{b}$ | 13.03 ± 5.50 | 25.35 ± 25.38 | 42.95 ± 10.25$^{a}$ |
| | | 60 | 50.45 ± 6.88$^{b}$ | 47.37 ± 6.32$^{b}$ | 7.45 ± 5.52 | 11.22 ± 6.84 | 35.07 ± 11.00 | 32.97 ± 10.87$^{b}$ | 1.45 ± 3.03$^{a}$ | 14.25 ± 4.72 | 26.70 ± 26.41 | 46.50 ± 9.76$^{a}$ |
| | N 96 | 40 | 46.17 ± 8.73$^{b}$ | 38.80 ± 8.70$^{a}$ | 4.62 ± 5.12 | 10.37 ± 6.98 | 30.75 ± 11.09 | 32.00 ± 9.75$^{a}$ | 2.07 ± 3.56$^{a}$ | 13.41 ± 5.21 | 24.40 ± 24.48 | 40.70 ± 10.78$^{a}$ |
| | | 50 | 44.77 ± 9.45$^{a}$ | 40.42 ± 8.25$^{b}$ | 6.12 ± 6.29 | 10.87 ± 7.40 | 30.87 ± 12.21 | 37.55 ± 9.76$^{b}$ | 1.32 ± 2.30$^{b}$ | 14.48 ± 5.04 | 25.62 ± 27.45 | 39.77 ± 12.05 |
| | | 60 | 49.17 ± 9.21$^{b}$ | 45.47 ± 13.75$^{b}$ | 5.32 ± 4.76 | 9.42 ± 7.52 | 34.20 ± 9.95 | 32.77 ± 11.17$^{b}$ | 1.55 ± 3.42$^{b}$ | 13.51 ± 5.21 | 25.25 ± 26.41 | 47.12 ± 9.64$^{b}$ |

[1]Results reported as mean ± standard deviation for 10 judges and 3 sessions. A multivariate ANOVA with Duncan's multiple comparison post-hoc tests was performed. $^{a, b}$ Means with different superscripts in each column are significantly different ($p < 0.05$) for pulp percentage. Means with different colours in each column are significantly different ($p < 0.05$) between sessions.

fermented beverage samples can cause both a bitter and astringent sensation, which are easily confused by panellists (Noble, 1999). Therefore, the influence of astringency in samples could have confounded the panel's response to bitterness (Noble, 1999). Further credence is lent to this hypothesis, since astringency was also not rated significantly different ($p > 0.05$) (Table 3.8). Hence, the processing parameters as described in section 3.3.2 resulted in alcoholic fermented plum beverages that were sensorially comparable to typical aroma, flavour, taste and mouthfeel attributes associated with white wines (Sokolowsky *et al*., 2015).

## 3.7 Conclusion

The study aimed to measure the dependent variables (DV) which constitute the key quality parameters for white wines (Nieuwoudt *et al*., 2002; Sahin & Sammu, 2006; Recemales *et al*., 2006; King *et al*., 2010; Louw *et al*., 2010; Sokolowsky *et al*., 2015), namely methanol, ethanol, titratable acidity, objective colour, total soluble solids, pH, sensory profile in response to two independent variables (ID), namely yeast strain and percentage pulp in order to adapt existing technologies towards producing an alcoholic fermented plum beverage based on white wine styles. From the results in this study it can be seen that the DV measured were similar to corresponding parameters of white wines. While the different treatments did not affect the sensory profile significantly (Figure 3.4), the alcoholic fermented plum beverage produced by adapting existing technologies had sensory properties similar to that of white wines (Sokolowsky *et al*., 2014). Hence, based on these parameters (ID and DV) applied in this study, in a further study the technology will be applied to develop red wine styled alcoholic fermented beverages with high overall consumer acceptability, using alternative fruit varieties (red-fleshed plums and selected berries).

## 3.8 References

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# CHAPTER 4

# THE DEVELOPMENT OF ALCOHOLIC FERMENTED BEVERAGES UTILIZING RED-FLESHED PLUMS AND SELECTED BERRIES

## 4.1 Abstract

The adaptation of existing technologies toward producing an alcoholic fermented plum beverage based on a red wine style was undertaken in this study. The Independent variables (ID) were yeast strains (1) *Saccharomyces cerevisiae* VIN13, (2) NT116 and (3) *Saccharomyces bayanus* N96, with formulations containing percentage pulp concentrations at (40%, 50% and 60%). The dependent variables (DV) constituted key quality parameters for red wines, namely methanol, ethanol, titratable acidity, objective colour, total soluble solids, pH and sensory profile. For alcoholic fermented red-fleshed plum beverages the titratable acidity (TA) at 40% ($m.m^{-1}$) pulp was significantly lower ($p < 0.05$) than the TA of the 60% ($m.m^{-1}$) pulp, while the TA value at 50% ($m.m^{-1}$) pulp was not significantly different ($p > 0.05$) from either 40% or 60% pulp. The pH range of alcoholic fermented plum beverages was similar to that of red wines produced in South Africa. The %ethanol (EtOH) ($v.v^{-1}$) ranged from 3.55 – 12.13, which were similar to red grape wines. The %EtOH ($v.v^{-1}$) and °Brix values corresponded, as the same pattern was observed, namely where the independent variable %pulp increased from 40 – 60, the dependent variables °Brix and %EtOH ($v.v^{-1}$) increased significantly ($p < 0.05$) between 40 and 50% and also between 50 and 60%. The toxicant methanol was not detected in any of the samples. Objective colour measured in the alcoholic fermented red-fleshed plum beverages was also similar to that of typical red wine. As was the case with alcoholic fermented red-fleshed plum beverages, the same pattern was observed for alcoholic fermented blackberry and blueberry beverages, namely the TA increased as the %pulp increased in formulations from 40 – 60%. The increase in %EtOH ($v.v^{-1}$) and °Brix values also corresponded with the increase in %pulp from 40 – 60%. The pH ranges measured for these samples were also typical to that of red wines (pH 3.5 – 3.7). The objective colour measured for alcoholic fermented blackberry and blueberry were also similar to that found in red wines. The overall sensorial profile made use of the uninstructed sorting method to classify and profile the alcoholic

fermented red-fleshed plum, blackberry and blueberry beverages with the use of a trained panel. It was established that the uninstructed sorting task was used successfully to identify, classify and profile different alcoholic fermented fruit beverage styles. The overall consumer acceptability results showed among the alcoholic fermented fruit beverages red-fleshed plum samples were “liked very much” by consumers with the exception of one sample treatment, while the blackberry and blueberry beverage samples were rated as “disliked slightly” by consumers. The outcome of the study showed that adapting existing technology can be used to produce alcoholic fermented red-plum, blackberry and blueberry beverages of which the key quality parameters and attributes are comparable to red wines.

## 4.2 Introduction

Wine made from red grapes is the most widely produced alcoholic fermented fruit beverage globally (Giuliani *et al*., 2011). However, berry fruits are also used for production of wine using the same method used in wine-making using grapes (Johnson & Gonzalez de Mejia, 2012). Hence, utilizing berries for the development of alcoholic fermented beverages is a good opportunity to explore the role that agro-processing plays in value addition.

Berry fruits, in particular blackberries and blueberries, have been broadly identified as an outstanding source of bioactive phenolic compounds including flavonoids, phenolic acids and tannins (Seeram, 2008) that both individually and synergistically may help protect against cardiovascular disease, cancer, inflammation, obesity, diabetes and other chronic diseases (Liu *et al*., 2000; Liu, 2007; Prior *et al*., 2008; Kraft *et al*., 2008; Shukitt-Hale *et al*., 2008). Berry fruits are also a rich source of anthocyanins which are water-soluble pigments responsible for the red, blue and purple colour (Miguel, 2011). In terms of value-addition, berry fruits are often converted into dried and canned products, or processed into jams, jellies, juices and sometimes wines (Rickman *et al*., 2007). A study by Heinonen *et al*. (1998) showed that wine made from berries can exhibit similar physicochemical and sensory properties to that of red grape wines (Heinonen *et al*., 1998).

Sensory interactions between compounds naturally present in wine have been widely demonstrated to occur in simple model solutions (Stevenson, 2010), as well as in more complex systems. Sensory evaluation comprises a set of techniques for

accurate measurements of human responses to foods and minimizes the potential bias effects of brand identity (Lawless & Heymann, 1998) and hence would be an important tool in a study evaluating the suitability of plums and berries as substrates for wine-making.

Also, the acceptance of a food will depend on whether it aligns with consumer needs and on the degree of satisfaction that it is able to provide (Heldman, 2004). Accordingly, consumer sensory testing is a commonly applied tool in research and product development. The main goal of consumer acceptability or preference study is to establish the relationship between preference and the degree of acceptance (Tenenhaus *et al*., 2005). The hedonic continuum is a frequently used expression for product liking and may be considered the more generic representation of the affective process.

Sorting is another sensory evaluation technique that was developed to simplify the panel response to complex food matrices such as alcoholic fermented beverages. A sorting technique is a relatively simple sensory evaluation method in which panellists are asked to examine samples and group them according to a similar property in terms of aroma, flavour, colour and others. The sorting procedure is based on categorization, a cognitive process naturally used in daily life that does not necessarily require a quantitative evaluation of the stimuli. The objective of the sorting task is to uncover the structure of the product space and to interpret the underlying dimensions *via* statistical analyses. The statistical interpretations of data collected are distance matrices that can be analysed using various sets of methods. Some of these techniques are DISTATIS (Abdi *et al.*, 2007) and correspondence analysis (CA) (Sinesio *et al.*, 2007). The sorting task has been proved to be predominantly well adapted for the evaluation of food products with the advantage of it being quicker and leading to slightly less fatigue and boredom, even though some short term memory problems can occur when a large number of products has to be evaluated (Patris *et al*., 2007; Chollet *et al*., 2011). The aforementioned sorting task and sorting data techniques has been used in studies that produced alcoholic fermented beverages such as beer (Chollet *et al*., 2011) and wine (Parr *et al*., 2010). Furthermore, the production of alcoholic fermented fruit beverages that are sensorially as acceptable as grape wine could develop niche markets leading to value-addition of underutilized produce, thus creating a market for high-value products that could ultimately create new economic opportunities for developing

countries like South Africa (Reardon *et al*., 2001). Moreover, the development of alcoholic fermented fruit beverages can potentially lead to more opportunities for smallholding and low income producers in South Africa and could also increase the industrial utilization of agricultural produce (fruit) presently going to waste in the fruit industry.

The aim of this study was to measure methanol, ethanol, titratable acidity, objective colour, total soluble solids and sensory profile as a function of yeast strain, pulp percentage and sugar levels in order to adapt existing technologies toward producing new fermented fruit beverages using red-fleshed plums, blueberries and blackberries.

## 4.3 Materials and methods

### *4.3.1 Preparation of alcoholic fermented beverages* using *red-fleshed plum and selected berries*

#### *4.3.1.1 Fruit preparations*

Two variants (PR04-36 and PR05-09) of red-fleshed plums were obtained from the Agricultural Research Council (Infruitec-Nietvoorbij, Stellenbosch, South Africa). Only mature fruits were included when selecting plums for use in this study. To control the ripening, plums received from the supplier were placed into 20 kg plastic fruit crates and placed into cold storage at (4˚C) for a maximum of two weeks. After storage, plums that were free from mould were thoroughly washed before cutting. Plums were cut in half by hand using stainless steel paring knives, after which the stones were removed to prepare the fruit for the pulping process. Pulping was achieved using a fruit-pulper fitted with a 2 mm stainless steel sieve (Jas Enterprises, Rakhial Ahmedabad, India). During the pulping process, the plum halves were fed slowly through the fruit pulper to prevent blockage and it also allowed peels to be separated from the pulp. The red-fleshed plum pulp was inoculated after pulping with selected yeasts towards production of alcoholic fermented red-fleshed plum beverages.

The selected berries (blackberries and blueberries) were obtained commercially from Hillcrest Berry Orchards (Stellenbosch, South Africa). The selected berries received from the supplier were sub-divided in 2 kg quantities and

packed into polyethylene bags after which it was placed in frozen storage at -15°C, making them available for use throughout the study. The selected berries were pulped using an Ultra-Turrax UTS (IKA®-Works Inc., Staufen, Germany) which ran at maximum speed until the pulp was formed. As before, the berry pulps were inoculated after pulping.

#### *4.3.1.2 Product*

The formulations used in this study were based on the formulations developed in Chapter 3 (Table 3.1) where the percentage pulp concentration ranged between 40 – 60%. In this study three sugar levels, targeting namely 6%, 8% and 10% EtOH ($v.v^{-1}$) were used. Three yeast strains namely VIN 13, N 96 and NT 116 were used in the fermentation of the must. The formulations for the alcoholic fermented red-fleshed plum and selected berries (blackberries and blueberries) beverages were recorded in Table 4.1.

### *4.3.2 Production of alcoholic fermented red-fleshed plum and berry beverages*

The three yeast strains that were selected and used in this study were: *Saccharomyces cerevisiae* hybrid VIN 13 (ester forming), NT 116 (RED) used for red wines, both from Anchor Yeast (Cape Town, South Africa) and *Saccharomyces bayanus* N 96 (N = Nuy wine cellar), (Anchor Yeast Cape Town, South Africa). The winemaking process used for the alcoholic fermented red-fleshed plum beverage was the same process previously described in Chapter 3 under Materials and Methods (Section 3.3.2). The winemaking process used for the alcoholic fermented berry beverages (blackberries and blueberries) deviated from the aforementioned methodology in terms of the omission of adding 1.2 mL pectolytic enzyme preparations and also that the bottled beverage samples were subjected to pasteurization in a heating tunnel at an air temperature of 80°C for 45 minutes, followed by cooling in water at 10°C.

**Table 4.1** Red-fleshed plum and berry alcoholic fermented beverage formulations

| **Percentage pulp** | **40% ($m.m^{-1}$)** | | | **50% ($m.m^{-1}$)** | | | **60% ($m.m^{-1}$)** | | |
|---|---|---|---|---|---|---|---|---|---|
| Potential %EtOH ($v.v^{-1}$) | 10 | 8 | 6 | 10 | 8 | 6 | 10 | 8 | 6 |
| Pulp (17.5°B[1] or 11.3°B[2]) added (kg) | 1.80 | 1.80 | 1.80 | 2.25 | 2.25 | 2.25 | 2.70 | 2.70 | 2.70 |
| Sugar added (kg) | 0.63 | 0.44 | 0.25 | 0.55 | 0.36 | 0.17 | 0.47 | 0.28 | 0.09 |
| Water added (kg) | 2.07 | 2.26 | 2.45 | 1.70 | 1.89 | 2.08 | 1.33 | 1.52 | 1.71 |
| Total mass (kg) | 4.50 | 4.50 | 4.50 | 4.50 | 4.50 | 4.50 | 4.50 | 4.50 | 4.50 |

[1]For red-fleshed plums the pulp was at 17.5°B, [2]For selected berries (blackberries and blueberries) the pulp was at 11.3°B.

### *4.3.3 Chemical analyses*

#### *4.3.3.1 Materials*

Unless otherwise specified, all the chemicals used in this study were of analar grade and chemical reagents were prepared according to standard analytical procedures.

#### 4.3.3.2 *All aspects of the Gas Chromatography assay (Linearity curve, Repeatability and Gas chromatography assay)*

The linearity curve for EtOH and MeOH was constructed using the method previously described in Chapter 3 under Materials and Methods (Section 3.3.3.2), while the repeatability or relative precision of the method was established as described in Section 3.3.3.3. The Gas chromatography assay for MeOH and EtOH in the beverage samples was performed using the method previously described in Chapter 3 under Materials and Methods (Section 3.3.3.4).

#### *4.3.3.3 Titratable acidity, Total soluble solids (TSS) and Spectrophotometric measurements*

The titratable acidity (TA) of the alcoholic fermented beverages was determined using the method previously described in Chapter 3 under Materials and Methods (Section 3.3.3.5), while the total soluble solids (TSS) content of the samples were measured in °Brix using the method described in Section 3.3.3.6. The CIELab colour co-ordinates, namely $L^*$, $a^*$, $b^*$, C and h of the alcoholic fermented beverage samples were measured using the method described in Section 3.3.3.7.

### *4.3.4 Sensory evaluation (Uninstructed sorting task)*

#### *4.3.4.1 Sensory panel composition*

Nine female judges and one male, ranging in age from 20 to 65 participated in the study. They were selected based on their availability and product interest.

#### *4.3.4.2 Panel training*

During the uninstructed sorting task training sessions the sensory panel was informed about the background and objectives of the study and instructed on the sensory evaluation procedure. They were instructed to remove the plastic cap from

the serving glass, swirl the glass three times in an anti-clockwise rotation and evaluate the aroma of the alcoholic fermented beverages. The sensory panel also used the wine aroma wheel as a guide to identify aroma descriptors in the beverage samples (Noble *et al*., 1987). Thereafter, they were instructed to evaluate the flavour, taste and mouthfeel, by sipping a mouthful of each fruit alcoholic fermented beverage. The panel was also instructed to cleanse their palate in-between samples using water and unflavoured water biscuits.

During the first part of training, sensory panellists were exposed to a number of alcoholic fermented beverage samples to familiarise themselves with the product and the analysis protocol. During 3 one-hour sessions, the 9 samples of each of red-fleshed plum and selected berry alcoholic fermented beverages were analysed and compared to one another. The panel generated descriptors developing terminology for sensory properties such as flavour, fragrance, aroma and mouthfeel. Aroma was defined as the sensory properties perceived through orthonasal analysis, while flavour referred to the retronasal perception in the mouth. The term ‘taste’ was used to describe the basic taste modalities, i.e. sweet, sour, salty and bitter. Mouthfeel was described as the tactile sensation that occurred in the oral cavity after sipping the beverage samples. Descriptive terms (or descriptors) were suggested and deliberated by the sensory panel members and each new term was recorded. The relationship amongst the terms was discussed and definitions as well as actual reference standards (Table 4.2) for the prevailing sensory descriptors were obtained. In each session the sensory panel were given 9 samples of each particular alcoholic fermented fruit beverage, each of which was obtained from the same batch throughout the sensory analysis period, thereby ensuring consistency in sensory attributes between samples.

During the second part of training for the uninstructed sorting, descriptors were generated for each of the alcoholic fermented fruit beverages. The sensory descriptors generated for alcoholic fermented red-fleshed plum beverage were sixteen (16) aroma and ten (10) flavour, taste and mouthfeel descriptors for the 9 samples; for alcoholic fermented blackberry beverages nine (9) aroma and eight (8) flavour, taste and mouthfeel descriptors were generated, while the sensory descriptors generated for alcoholic fermented blueberry were ten (10) aroma and eight (8) flavour, taste and mouthfeel descriptors for the 9 samples. These terms

**Table 4.2** Aroma attributes and reference standards presented to the sensory panel during panel training sessions

| Aroma attributes | Physical standards supplied to the panel |
|---|---|
| **Plum** | Plum slices in 100 mL water & 100 mL of red fleshed plum alcoholic fermented beverage. |
| **Blackberry** | 100 g of blackberries in 100 mL water & 100 mL of blackberry alcoholic fermented beverage. |
| **Blueberry** | 100 g of blueberries in 100 mL water & 100 mL of blueberry alcoholic fermented beverage. |

**Table 4.3** Aroma (A), flavour (F), taste (T) and mouthfeel (MF) sensory attributes selected by the panel for the uninstructed sorting task

| Fruit beverage | Aroma attributes | Flavour, taste and mouthfeel attributes |
|---|---|---|
| **Red-Fleshed plum** | Yeasty (A) | Plum (F) |
| | Musty (A) | Fruity (F) |
| | Fruity (A) | Berry (F) |
| | Plum (A) | Sweet (T) |
| | Sweet (A) | Bitter (T) |
| | Banana (A) | Sour (T) |
| | Berry (A) | Astringent (MF) |
| | Candy (A) | |
| | High alcohol (A) | |
| **Blackberry** | Fruity (A) | Fruity (F) |
| | Berry (A) | Berry (F) |
| | Banana (A) | Muscadel (F) |
| | Blackberry (A) | Sherry (F) |
| | Muscadel (A) | Plum (F) |
| | Sherry (A) | Raisin (F) |
| | Sweet (A) | High alcohol (T) |
| | Raisin (A) | Bitter (T) |
| | Plum (A) | Sour (T) |
| | Dusty (A) | Astringent (MF) |
| | Yeasty (A) | Lingering aftertaste (MF) |
| | High alcohol (A) | |
| | Chemical (A) | |
| **Blueberry** | Fruity (A) | Fruity (F) |
| | Floral (A) | Floral (F) |
| | Banana (A) | Berry (F) |
| | Berry (A) | Green grass (F) |
| | Sweet (A) | Earthy (T) |
| | Musty (A) | Yeasty (T) |
| | Earthy (A) | High alcohol (T) |
| | Green grass (A) | Sweet (T) |
| | High alcohol (A) | Bitter (T) |
| | Chemical (A) | Sour (T) |
| | | Astringent (MF) |

were selected for the inclusion in the uninstructed sorting task based on their frequency of being monitored by the panel during the training phase (Table 4.3). In each session each panellist was given 9 samples of each fruit alcoholic fermented beverage.

#### *4.3.4.3 Testing procedure*

The entire uninstructed sorting task consisted of two sessions and took less than 1 hour to complete. The alcoholic fermented red-fleshed plum and selected berry beverage samples were served at ambient temperature (21°C) in standard ISO wine tasting glasses covered with small plastic caps. Each panellist received 30 mL of each sample. The alcoholic fermented beverages were labelled from 1 – 9 and served in a randomised order.

During the first session of the uninstructed sorting task, the sensory panel was given information about the samples presented which indicated that, for each fruit variety, the samples before them represented 3 different yeast strains (VIN 13, N 96 and NT 116), 3 different pulp concentrations (40%, 50%, 60%) and 3 different sugar levels projected to yield %EtOH ($v.v^{-1}$) at 6%, 8%, 10%, respectively. Each alcoholic fermented fruit beverage variety consisted of 9 samples. The panel was asked to group the samples according to the similarity of their aroma profiles. During the uninstructed sorting task, the sensory panel was allowed to smell the samples numerous times. Thereafter, on a blank A3 page that was provided, panellists were allowed to group together the samples that had similar aroma profiles. However, they were limited to sorting into a maximum of five groups. Panellists then used an evaluation form provided on a separate A4 page and were allowed to indicate which samples they had grouped together. They were instructed to write down the major aroma attributes associated with each of the sample groups and were told to not exceed 5 attributes and to describe the aroma characteristics of each group.

For the second session of the uninstructed sorting task, panel was given information about the samples presented which indicated that, for each fruit variety, the samples before them represented 3 different yeast strains, 3 different pulp concentrations and 3 different sugar levels, as described in the previous paragraph.

As before, each alcoholic fermented fruit beverage variety consisted of 9 samples. The panel was asked to group the samples according to the similarity of their flavour profiles. During the uninstructed sorting task, the sensory panel was

allowed to sip the samples numerous times. Thereafter, on a blank A3 page that was provided, panellists were allowed to group together the samples that had similar flavour profiles. However, as before they were limited to five groups. Panellists then used an evaluation form provided on a separate A4 page and were allowed to indicate which samples they had grouped together. As before, they were instructed to write down the major flavour attributes associated with each of the sample groups and were told to not exceed 5 attributes and to describe the flavour characteristics of each group.

### *4.3.5 Consumer acceptance sensory evaluation*

The consumer acceptance sensory evaluation was conducted in the Sensory Evaluation facility at the Food Technology Department at Cape Peninsula University of Technology (CPUT), Bellville, South Africa.

#### *4.3.5.1 Sensory panel composition*

Twenty five consumers consisting of staff and students aged between 20 and 55 from (CPUT). The consumers were selected based familiarity with similar products (grape wine and wine coolers).

#### *4.3.5.2 Testing procedure*

Each panellist was served with a set of 9 samples of alcoholic fermented fruit beverage (red-fleshed plum, blackberry or blueberry), each sample (30 mL) was identified by a three-digit random number on an evaluation form used in the department (See example – Figure 4.1) The panellists were required to rate the overall taste of each sample. The rating was on a nine-point numerical hedonic scale labelled from 1 – Dislike extremely to 9 – Like extremely.

#### *4.3.5.3 Samples and sample serving*

Samples (30 mL) were served at refrigerated temperature (4$^{\circ}$C) in 50 mL clear plastic sample cups accompanied with 2 palate cleansers (crackers) and spittoon cup. Each panellist was also instructed to take a sip of water and eat a piece of cracker after they tasted each sample. This was done to cleanse the palate thereby avoiding carry-over effects in-between samples.

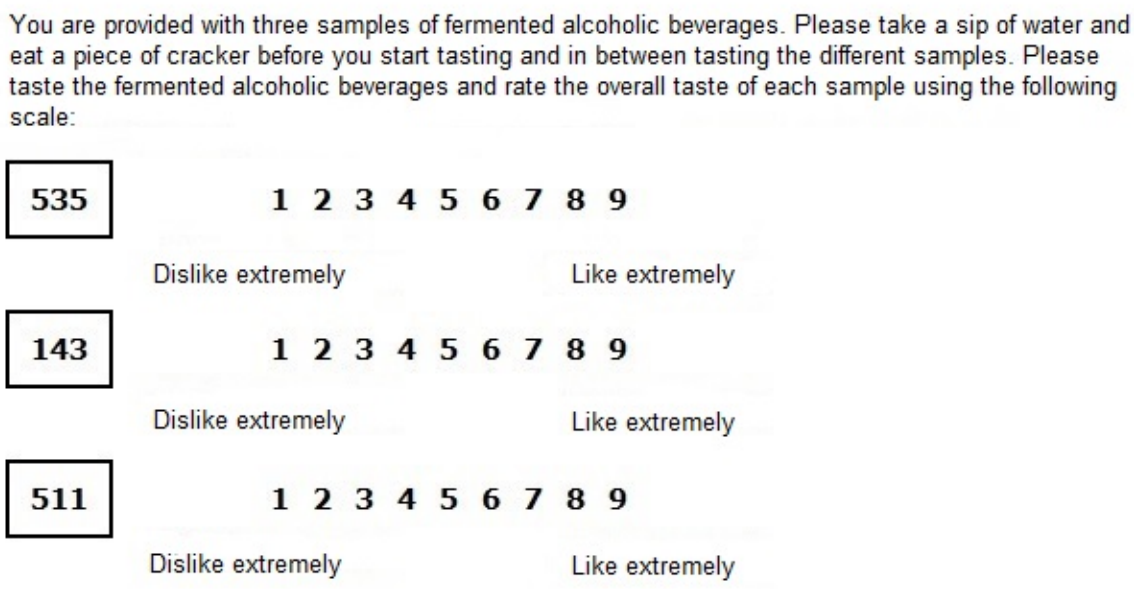


**Figure 4.1** Representation of the evaluation form used during the consumer acceptability sensory evaluation, showing a nine-point numerical hedonic scale

### *4.4 Experimental design*

The physicochemical experimental design was a 3 x 3 x 3 factorial design with three pulp concentrations (40%, 50%, 60%), three yeast strains VIN 13, N 96 and NT 116 and three sugar levels (equivalent to 6%, 8% and 10% EtOH ($v.v^{-1}$)). The response variables were titratable acidity (grams malic acid per litre), $^{\circ}$Brix, pH, ethanol (EtOH ($v.v^{-1}$)), methanol (MeOH ($v.v^{-1}$)) and colour ($L^*$, $a^*$, $b^*$, C, h). For the uninstructed sorting task, ten trained sensory panellists were used. The response variables were the sensory attributes (aroma and flavour; Table 4.3) that was generated during the uninstructed sorting of alcoholic fermented beverages produced in the study.

### *4.5 Data analyses*

The physicochemical data and consumer acceptability sensory evaluation data was subjected to a multivariate analysis of variance (ANOVA) to ascertain whether the main effects resulted in significant differences in response variables. The Duncan's multiple comparison post hoc test was used to test significant differences ($p < 0.05$) between individual means, using IBM® SPSS® statistical software (Version 22; IBM Corporation, New York, USA). For the uninstructed sorting task each panellist's distance matrix was converted into a covariance matrix which compared the pattern for each alcoholic fermented fruit beverage directly with all the other alcoholic fermented fruit beverages sorted by the panel in each sample set. The data was processed using DISTATIS and correspondence analysis (CA). All analyses were conducted using Statistica software 10 (StatSoft, Inc.).

DISTATIS took into account individual sensory data as it is performed directly on individual distance matrices, which is a three-way generalization of classical multidimensional scaling which provides a map of the samples. This map is known as the "compromise map" which integrates the panels' distance matrices in the most efficient way. In this map, the proximity between sample points reflected there similarity. Overall, DISTATIS provided information about the panellists' agreement because it also shows how each panelist positioned the samples relative to the compromise map (Abdi *et al*., 2005; Abdi, 2007).

CA visualized the relationship between samples and sensory descriptors. Using this technique row and column variables were spatially represented, which allowed a visual representation of the data (Ten Kleij & Musters, 2003). CA is a

descriptive technique, designed to analyse simple two-way correlation tables containing some measure of correspondence between the rows and columns.

## *4.6 Results and Discussion*

### *4.6.1 Linearity*

The linearity curve for the specific MeOH and EtOH peak areas and concentrations of the injected standards over a range of concentrations were reported and discussed in detail in Chapter 3, Section 3.6.1. In summary, for MeOH the concentration of the injected standards ranged between 0.5, 1, 2, 3 and 4% ($v.v^{-1}$). The regression coefficient (R) for MeOH was 0.99972 and the correlation coefficient ($R^2$) = 0.99946. The LOD for MeOH was 0.00000142 $\mu g.mL^{-1}$ and the LOQ was 0.0000473 $\mu g.mL^{-1}$.

For EtOH, the concentration of the injected standards over a range of concentrations ranged between 5, 10, 15, 20 and 25% ($v.v^{-1}$). The regression coefficient (R) for EtOH was 0.99967 and the correlation coefficient ($R^2$) = 0.99934. The LOD was 0.000603 $\mu g.mL^{-1}$ and the LOQ was 0.00201 $\mu g.mL^{-1}$. The results for both MeOH and EtOH confirmed that the concentration range of interest over five points having equal spacing showed a suitable calibration pattern, while the high correlation co-efficients of > 0.999 were evidence of good linear fit. The calibration results for both MeOH and EtOH complied with the AOAC guidelines (Anon., 2002).

### *4.6.2 Precision*

The repeatability or relative precision for MeOH and EtOH was reported and discussed in detail in Chapter 3, Section 3.6.2. In summary, the method used for relative precision measured five replicates per session of standard solutions of known concentrations for MeOH and EtOH over two consecutive sessions on the same day and over two consecutive days, i.e. intra-day and inter-day assays. The measured concentrations for MeOH and EtOH between sessions (intra-day) and (inter-day) on separate days were not significant ($p > 0.05$) in all cases, indicating acceptable precision of the analytical methods.

### *4.6.3 Physicochemical analyses*

#### *4.6.3.1 Statistical interaction between independent variables for all beverage variants*

For all three beverage variants, the statistical results for the interactions between the independent variables, namely yeast (VIN 13, N 96 and NT 116) and %pulp (40, 50 and 60) in terms of the response (dependent) variables (Titratable acidity (TA), TSS (°Brix), pH, Ethanol (EtOH) ($v.v^{-1}$), Methanol (MeOH) ($v.v^{-1}$) and CIELab colour coordinates L*, a*, b*, C, and h) showed no significance ($p > 0.05$). Therefore, the effect of each of the two independent variables on the response variables was not confounded by the other. These results were not depicted in Tables 4.5 – 4.7.

#### *4.6.3.2 Methanol (MeOH) levels*

MeOH was not detected in the present study (Table 4.5 – 4.7), which means that the MeOH was either absent or present in levels less than 0.00000142 $\mu g.mL^{-1}$, the LOD for MeOH, in all three beverage variants included in the study. This is ideal because MeOH is commonly considered to be an undesirable constituent of alcoholic fermented beverages. Furthermore, MeOH is also known to be toxic to humans when consumed even in low concentrations (Campos *et al*., 2010).

#### *4.6.3.3 Alcoholic fermented red-fleshed plum beverages*

The results confirmed that the independent variable yeast strain (VIN 13, N 96 and NT 116) did not have a significant ($p > 0.05$) effect on any of the response variables (Table 4.4). The results of the multivariate Analysis of Variance (MANOVA) indicated that %pulp (40, 50 and 60) had a significant effect on the response variables ($p < 0.05$). However, the post hoc test showed that significant differences ($p < 0.05$) were found for Titratable acidity (TA), TSS (°Brix), pH and EtOH ($v.v^{-1}$), while MeOH ($v.v^{-1}$) and CIELab colour coordinates (L*, a*, b*, C, and h) showed differences that were not significant ($p > 0.05$) (Table 4.4).

The means for TA ranged from 8.47 – 11.28 (Table 4.4), with the TA of the 40% ($m.m^{-1}$) pulp significantly lower ($p < 0.05$) than the TA of the 60% ($m.m^{-1}$) pulp, while the TA value at 50% ($m.m^{-1}$) pulp was not significantly different ($p > 0.05$) from either 40% or 60% pulp. The TA values measured for the alcoholic fermented red-

**Table 4.4** Physicochemical profile of alcoholic fermented red-fleshed plum beverage samples

| Treatments | Chemical Profile | | | | | Physical profile | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Titratable acidity (TA) as malic acid ($g.L^{-1}$) | Brix (°B) | pH | EtOH ($v.v^{-1}$) | MeOH ($v.v^{-1}$) | L* | a* | b* | C | h |
| **%Pulp** | | | | | | | | | | |
| **40** | 9.40 ± 1.84[a] | 3.30 ± 0.30[a] | 3.41 ± 0.02[a] | 3.55 ± 0.20[a] | n.d.[2] | 87.53 ± 5.10 | 19.02 ± 8.51 | 2.01 ± 1.20 | 18.56 ± 8.23 | 4.99 ± 1.48 |
| **50** | 8.47 ± 1.95[a, b] | 5.53 ± 0.19[b] | 3.27 ± 0.04[b] | 5.75 ± 0.50[b] | n.d. | 84.60 ± 2.80 | 23.34 ± 4.13 | 3.03 ± 1.04 | 22.97 ± 4.34 | 6.80 ± 1.93 |
| **60** | 11.28 ± 1.18[b] | 8.26 ± 0.35[c] | 3.31 ± 0.08[b] | 12.13 ± 0.51[c] | n.d. | 83.21 ± 1.61 | 23.69 ± 1.50 | 3.23 ± 0.77 | 23.90 ± 1.57 | 7.70 ± 1.48 |
| **Yeast** | | | | | | | | | | |
| **VIN 13** | 8.69 ± 1.65 | 5.61 ± 2.03 | 3.35 ± 0.11 | 7.17 ± 3.81 | n.d.[2] | 86.03 ± 2.44 | 21.21 ± 3.93 | 2.49 ± 0.73 | 20.80 ± 3.88 | 6.16 ± 1.65 |
| **N 96** | 10.73 ± 1.98 | 5.71 ± 2.41 | 3.32 ± 0.07 | 7.14 ± 4.19 | n.d. | 85.80 ± 5.35 | 20.70 ± 8.16 | 2.55 ± 1.36 | 20.64 ± 8.15 | 6.20 ± 2.42 |
| **NT 116** | 9.72 ± 2.09 | 5.76 ± 2.27 | 3.31 ± 0.08 | 7.11 ± 4.00 | n.d. | 83.57 ± 3.01 | 24.14 ± 4.19 | 3.23 ± 1.16 | 23.99 ± 4.31 | 7.14 ± 1.83 |

[1]Results reported as mean ± standard deviation a multivariate analysis of variance (ANOVA) with Duncan's multiple comparison post-hoc test was performed and [a – c] Means with different letters superscripts in each column are significantly different ($p < 0.05$). [2]n.d. = not detected.

fleshed plum beverages produced in this study were higher than the total acidity ($g.L^{-1}$ tartaric acid) measured in red wines of which the typical average ranged from 5.2 – 5.36 (Lisanti *et al*., 2013). However, since tartaric acid is more tart than malic acid (Fahmi *et al*., 2013), it can be considered comparable.

The TSS measured in °Brix values were found to be significantly different ($p < 0.05$) for each pulp concentration level (40, 50 and 60%) (Table 4.4), with the °Brix value increasing as the pulp concentration increased from 40% to 50% and from 50% to 60% pulp concentration. These results for °Brix were expected since a progressively higher %pulp was used in the formulations that were used to produce the alcoholic fermented red-fleshed plum beverages in this study.

The pH average values recorded for %pulp decreased significantly ($p < 0.05$) as the %pulp was increased from 40% (3.41) to 50% (3.27), while the increase in pH between 50% and 60% pulp was not significant ($p > 0.05$) (Table 4.4). Furthermore, the pH range of the red-fleshed plum alcoholic fermented beverages produced in this study was similar to the average pH ranges of South African red wines reported by Du Toit & Lambrechts (2002), where the pH ranged from 3.41 – 3.76. Moreover, the results for pH followed a similar pattern as did the TA, namely the pH decreased significantly ($p < 0.05$) as the %pulp increased from 40% to 50%, while the pH values at 50% and 60% were not significantly different ($p > 0.05$) (Table 4.4).

The %EtOH ($v.v^{-1}$) ranged from 3.55 – 12.13 (Table 4.4), with the %EtOH ($v.v^{-1}$) significantly different ($p < 0.05$) for each %pulp concentration level and the %EtOH ($v.v^{-1}$) increasing significantly ($p < 0.05$) as the %pulp concentration increased from 40 to 50% and from 50 to 60%. Furthermore, the %EtOH ($v.v^{-1}$) levels measured for the alcoholic fermented red-fleshed plum beverages produced in this study were similar to red grape wines reported by Lago-Vanzela *et al*. (2013), where the average %EtOH ($v.v^{-1}$) levels ranged from 8 – 12, but were even more comparable to ready-to-drink products such as wine coolers where the average %EtOH ($v.v^{-1}$) levels ranged from 5 – 10 (Hu, 2012). The results for %EtOH ($v.v^{-1}$) and °Brix values corresponded, as the same pattern was observed, namely where the independent variable %pulp increased from 40 – 60, the dependent variables °Brix and %EtOH ($v.v^{-1}$) increased significantly ($p < 0.05$) between 40 and 50% and also between 50 and 60% (Table 4.4).

The colour of the alcoholic fermented red-fleshed plum beverage samples measured were based on the CIELab colour coordinates, namely L*, a*, b*, C, and

h. Colour values were expressed as L* (whiteness or brightness/darkness, a* (redness/greenness), b* (yellowness/blueness), Chroma C was calculated from $(a^2 + b^2)^{½}$, where the zero value represented black and 100 represented white. The hue angle h represented colour by a positive number 0° and 360°, where 0° = reddish-purple, 90° = yellow, 180° bluish-green and 270° = blue which was in essence a measure of angular deviation of the sample colour from the three primary colours of red, yellow and green. The average range of each CIELab colour coordinate treatments were, L* = 83.21 – 87.53, a* = +19.02 – +23.69, b* = +2.01 – +3.23, C = 18.56 – 23.90 and h = 4.99 – 7.70. The average range of CIELab colour coordinates typical for red wines produced in a study by Hayasaka *et al*. (2007), were L* = 72.19, a* = +27.61, b* = +5.19, C = 49.90, h = 0.76. The most noticeable differences between these CIELab colour space coordinates and the CIELab colour space coordinates measured for red-fleshed plum beverages produced in this study, were the higher values for L* (whiteness or brightness/darkness), higher positive values for a* (redness), while positive for b* (yellowness) values were closely correlated and higher values for both C and h were observed. Therefore, when comparing the CIELab colour coordinates of alcoholic fermented red-fleshed plum beverages produced in this study to that of red wines it is clear that the two types of alcoholic fermented beverages are similar in terms of colour. As a result, the processing parameters developed in this study resulted in alcoholic fermented red-fleshed plum beverages that were comparable to red wines in terms of the aforementioned physicochemical parameters.

#### *4.6.3.4 Alcoholic fermented blackberry beverages*

The results confirmed that the independent variable yeast strain (VIN 13, N 96 and NT 116) did not have a significant ($p > 0.05$) effect on the response variables (Table 4.5).

The results of the MANOVA indicated that %pulp (40, 50 and 60) had a significant effect on the response variables ($p < 0.05$). However, the post hoc tests showed that significant differences ($p < 0.05$) were found for Titratable acidity (TA), TSS ($^{\circ}$Brix), EtOH ($v.v^{-1}$) and CIELab colour coordinates (L*, a*, b*, C, and h), while the pH values did not change significantly ($p > 0.05$) in response to changes in %pulp (Table 4.5).

**Table 4.5** Physicochemical profile of alcoholic fermented blackberry beverage samples

| Treatments | Chemical profile | | | | | Physical profile | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Titratable acidity (TA) as malic acid ($g.L^{-1}$) | Brix (°B) | pH | EtOH ($v.v^{-1}$) | MeOH ($v.v^{-1}$) | L* | a* | b* | C | h |
| **%Pulp** | | | | | | | | | | |
| **40** | 10.63 ± 0.95[a] | 2.93 ± 0.16[a] | 3.40 ± 0.02 | 4.21 ± 0.23[a] | n.d.[2] | 91.50 ± 1.44[a] | 7.55 ± 1.79[a] | 4.49 ± 1.63[a] | 9.42 ± 1.71[a] | 35.60 ± 10.24[a] |
| **50** | 12.33 ± 0.83[b] | 5.46 ± 0.15[b] | 3.34 ± 0.15 | 7.15 ± 0.37[b] | n.d. | 84.14 ± 2.10[a] | 13.29 ± 2.41[b] | 14.66 ± 1.39[b] | 18.86 ± 3.24[b] | 42.33 ± 15.18[a, b] |
| **60** | 13.48 ± 1.32[b] | 8.06 ± 0.33[c] | 3.22 ± 0.03 | 15.12 ± 0.49[c] | n.d. | 82.73 ± 5.66[b] | 14.41 ± 5.17[b] | 19.70 ± 6.17[b] | 24.43 ± 7.97[b] | 54.66 ± 3.70[b] |
| **Yeast** | | | | | | | | | | |
| **VIN 13** | 12.59 ± 1.75 | 5.48 ± 2.19 | 3.30 ± 0.11 | 9.06 ± 5.17 | n.d. | 85.54 ± 5.16 | 12.02 ± 4.22 | 14.63 ± 7.70 | 8.52 ± 8.42 | 45.08 ± 15.81 |
| **N 96** | 11.38 ± 1.36 | 5.46 ± 2.32 | 3.33 ± 0.10 | 8.70 ± 5.17 | n.d. | 86.79 ± 6.66 | 11.31 ± 6.04 | 11.23 ± 7.68 | 6.29 ± 9.16 | 44.06 ± 11.22 |
| **NT 116** | 12.46 ± 1.50 | 5.51 ± 2.39 | 3.34 ± 0.14 | 8.72 ± 4.82 | n.d. | 86.03 ± 4.45 | 11.93 ± 3.69 | 13.00 ± 7.83 | 7.09 ± 7.57 | 43.44 ± 13.98 |

[1]Results reported as mean ± standard deviation a multivariate analysis of variance (ANOVA) with Duncan's multiple comparison post-hoc test was performed and [a – c] Means with different letters superscripts in each column are significantly different ($p < 0.05$). [2]n.d. = not detected.

The average values for TA increased significantly ($p < 0.05$) as the %pulp increased from 40% (10.63) to 50% (12.33), while the increase in TA between 50% and 60% pulp was slightly higher with differences not significant ($p > 0.05$) (Table 4.5).

The °Brix values were found to be significantly different ($p < 0.05$) for each pulp concentration level (40%, 50% and 60%) (Table 4.5), with the °Brix value increasing as the pulp concentration increased from 40% to 50% and from 50% to 60% pulp concentration. These results were expected since a higher pulp concentration signifies a higher sugar concentration.

The average pH values ranged from 3.22 – 3.40 with differences not significant ($p > 0.05$) (Table 4.5). However, the pH values measured for alcoholic fermented blackberry beverages produced in this study were still comparable to red wines reported by Walker & Blackmore (2012), where the pH ranged from 3.5 – 3.7. Furthermore, the pH of the alcoholic fermented blackberry beverages produced in this study were also comparable to alcoholic fermented blackberry beverages produced in a studies by Johnson & Gonzalez de Mejia (2012) and  who reported an average pH that ranged from 3.1 – 3.7.

The %EtOH ($v.v^{-1}$) was found to be significantly different ($p < 0.05$) for each %pulp concentration level, increasing significantly ($p < 0.05$) from 40 to 50% and from 50 to 60% pulp concentration (Table 4.5). The %EtOH ($v.v^{-1}$) ranged from 4.21 – 15.12 which, for 40 and 50% pulp was comparable to the %EtOH ($v.v^{-1}$) found for red wines that ranged from 5 – 10 %EtOH ($v.v^{-1}$) (Hu, 2012). However the %EtOH ($v.v^{-1}$) for 60% was well above this range (Table 4.5).

The alcoholic fermented blackberry beverage samples were observed to be “red” in colour (Table 4.5). The results of the CIELab colour coordinates clearly showed that the value for L* ranged from 82.73 – 91.50, with the value for L* at 60% pulp concentration significantly lower ($p < 0.05$) than those for both 40% and 50% pulp concentration, while the value for L* for 40% and 50% pulp was non-significantly higher ($p > 0.05$) than for 50% pulp. The values found for the colour coordinate a* ranged from +7.55 – +14.41 with the value for a* significantly lower ($p < 0.05$) for 40% pulp concentration and non-significantly higher ($p > 0.05$) between 50% and 60% pulp concentration. The values for colour coordinate b* ranged from +4.49 – +19.70 and was also significantly lower ($p < 0.05$) for 40% pulp concentration and non-significantly higher ($p > 0.05$) between 50% and 60% pulp

concentration, The same applies to the C-values which that is the same pattern as observed for the a* value (Table 4.5). The values for colour coordinate h ranged from 35.60 – 54.66 (Table 4.5), with the h for 40% pulp concentration significantly lower ($p < 0.05$) than the h at 60% pulp concentration, while the h value at 50% pulp concentration was not significantly different ($p > 0.05$) from either 40 and 60 %pulp concentration. Furthermore, the CIELab colour coordinates of a typical red wine measured in a study done by Hayasaka *et al*. (2007) were L* = 69.31 – 72.19, a* = +27.61 – +29.64 and b* = +5.19 – +14.42. The most notable differences between these and the measurements for the blackberry beverage in this study were high values for L*, comparatively low positive values for a* and similar values for b*. Thus it can deduced that red wines have a more intense red colour compared to the alcoholic fermented blackberry beverage (Hayasaka *et al*., 2007; Ortiz *et al*., 2013). Therefore, when comparing the CIELab colour coordinates of alcoholic fermented blackberry beverages produced in this study to that of red wines it is clear that the two types of alcoholic beverages are comparable in terms of colour. Hence, the processing parameters described in detail in Chapter 3, Section 3.3.2 resulted in alcoholic fermented blackberry beverages that were comparable to red wines in terms of all the aforementioned physicochemical parameters.

#### *4.6.3.5 Alcoholic fermented blueberry beverages*

The results confirmed that the independent variable yeast strain (VIN 13, N 96 and NT 116) did not have a significant ($p > 0.05$) effect on the response variables (Table 4.6).

The results of the MANOVA indicated that %pulp (40, 50 and 60) had a significant ($p < 0.05$) effect on the response variables. However, the post hoc test showed that significant differences ($p < 0.05$) were found for (Titratable acidity (TA), TSS ($^{\circ}$Brix), EtOH ($v.v^{-1}$) and CIELab colour coordinates (L*, a*, b*, C, and h), while the pH values did not change significantly ($p > 0.05$) in response to changes in the %pulp (Table 4.6).

The average values for TA measured were found to be significantly different ($p < 0.05$) at all pulp concentration levels (Table 4.6), with the TA value increasing as the pulp concentration increased from 40% to 50% and from 50% to 60%. The decrease in TA values corresponded with non-significant ($p > 0.05$) decreases in the corresponding pH values (Table 4.6).

**Table 4.6** Physicochemical profile of alcoholic fermented blueberry beverage samples

| Treatments | Chemical profile | | | | | Physical profile | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| %Pulp | Titratable acidity (TA) as malic acid ($g.L^{-1}$) | Brix (°B) | pH | EtOH ($v.v^{-1}$) | MeOH ($v.v^{-1}$) | L* | a* | b* | C | h |
| **40** | 8.77 ± 0.29[a] | 3.00 ± 0.08[a] | 3.02 ± 0.08 | 4.30 ± 0.26[a] | n.d.[2] | 86.52 ± 1.45[b] | 14.37 ± 1.73[a] | 3.45 ± 0.30[a] | 14.78 ± 1.74[a] | 13.58 ± 1.04[a] |
| **50** | 9.82 ± 1.14[b] | 5.08 ± 0.34[b] | 2.98 ± 0.07 | 7.08 ± 0.64[b] | n.d. | 83.83 ± 6.66[b] | 17.41 ± 6.82[a] | 4.52 ± 2.96[a] | 17.88 ± 7.40[a] | 13.75 ± 2.76[a] |
| **60** | 11.34 ± 0.72[c] | 8.20 ± 0.37[c] | 2.93 ± 0.02 | 11.53 ± 0.67[c] | n.d. | 76.03 ± 3.71[a] | 25.07 ± 4.40[b] | 10.10 ± 1.01[b] | 27.03 ± 4.40[b] | 22.19 ± 2.05[b] |
| **Yeast** | | | | | | | | | | |
| **VIN 13** | 9.74 ± 1.31 | 5.21 ± 2.23 | 2.99 ± 0.07 | 7.72 ± 3.10 | n.d. | 82.73 ± 7.22 | 17.96 ± 7.41 | 6.28 ± 3.63 | 19.08 ± 8.10 | 18.10 ± 5.17 |
| **N 96** | 9.78 ± 1.30 | 5.53 ± 2.33 | 2.94 ± 0.06 | 7.82 ± 3.26 | n.d. | 82.60 ± 5.55 | 18.63 ± 5.93 | 5.74 ± 3.92 | 19.57 ± 6.85 | 15.64 ± 5.25 |
| **NT 116** | 10.41 ± 1.46 | 5.53 ± 2.47 | 3.00 ± 0.08 | 7.37 ± 3.52 | n.d. | 81.05 ± 6.81 | 20.26 ± 6.94 | 6.06 ± 3.42 | 21.04 ± 7.71 | 15.77 ± 3.50 |

[1]Results reported as mean ± standard deviation a multivariate analysis of variance (ANOVA) with Duncan's multiple comparison post-hoc test was performed and [a – c] Means with different letters superscripts in each column are significantly different ($p < 0.05$). [2]n.d. = not detected.

The °Brix values were found to be significantly different ($p < 0.05$) for each pulp concentration level (Table 4.6), with the °Brix value increasing as the pulp concentration increased from 40% to 50% and from 50% to 60%. These results with respect to °Brix were expected since a higher %pulp signified a higher sugar concentration.

The pH values ranged from 2.93 – 3.02 (Table 4.6), but were not significantly different ($p > 0.05$). However, the pH range measured in the alcoholic fermented blueberry beverages produced in this study were comparable to the pH range of red wines produced in a study by Walker & Blackmore (2012), where the pH ranged between 3.5 – 3.7, as well as alcoholic fermented blueberry beverages produced in a study by Johnson *et al*. (2012), where the pH ranged from 2.8 – 3.7.

The %EtOH ($v.v^{-1}$) was found to be significantly different ($p < 0.05$) for each %pulp concentration level, increasing significantly ($p < 0.05$) from 40 to 50% and from 50 to 60% pulp concentration (Table 4.6). Hence, the results for %EtOH and °Brix corresponded, as the same pattern was observed. This was expected, since increases in %pulp from 40 to 50% and from 50 to 60% signified increases in sugar concentration.

The alcoholic fermented blueberry beverage samples were observed to be “red” in colour. The results (Table 4.6) showed that there were no significant differences ($p < 0.05$) between 40% pulp and 50% pulp in terms of all CIELab colour space coordinates L*, a*, b*, C and h, while the values for 60% pulp were significantly different ($p < 0.05$) (Table 4.6). The ranges of all the CIELab colour coordinates for all treatments were L* = 76.03 – 86.52, a* = +14.37 – +25.07, b* = +3.45 – +10.10, C = 14.78 – 27.03 and h = 13.58 – 22.19. The CIELab colour coordinates of red wines measured in an aforementioned study reported by Hayasaka *et al*. (2007) in blackberry beverages were also compared with the alcoholic fermented blueberry beverages. The most notable differences between CIELab colour coordinate measurements between red wines and the blueberry beverage samples in this study were higher values for L*, lower positive values for a* and similar positive values for b* (Hayasaka *et al*., 2007), thus a more intense red colour in the red wine samples compared to the alcoholic fermented blueberry beverage samples. Therefore, when comparing the aforementioned CIELab colour coordinates of alcoholic fermented blackberry (previous section) and blueberry beverages produced in this study to that of red wines it is clear that the three types of

alcoholic beverages are similar in terms of colour. Hence, the processing parameters described in detail in Chapter 3, Section 3.3.2 resulted in alcoholic fermented blueberry and blackberry beverages that were comparable to red wines in terms of all the aforementioned physicochemical parameters.

### *4.6.4 Sensory evaluation*

#### *4.6.4.1 The application of uninstructed sorting for the sensory profiling of alcoholic fermented red-fleshed plum, blackberry and blueberry beverages*

During the uninstructed sorting task, the sensory panel sorted each alcoholic fermented beverage into a maximum of 5 groups. The samples represented the effects of the design variables, namely yeast strain (VIN 13, N 96 and NT 116), and pulp concentration (40%, 50% and 60%, projected to yield a %EtOH ($v.v^{-1}$) at 6%, 8% and 10%, respectively) on the sensory attributes (aroma and flavour). The statistical analytical techniques of DISTATIS and Correspondence analysis (CA) were used to analyse the data.

#### *4.6.4.2 Evaluating the sensory response for **aroma and flavour** for red-fleshed plum beverages using DISTATIS and CA plots*

The DISTATIS plots for both aroma and flavour for the red-fleshed plum beverages are not shown here, since they showed different groupings to the CA plots, which signified the possibility that the panel could not clearly distinguish between the different sample treatments. Since the CA plots depict both the sensory attributes and the groupings, these were included (Figures 4.2 and 4.3).

It can be seen in Figure 4.2 that the aroma attribute yeasty was strongly associated with the treatments NT 116, 50% pulp and VIN 13, 40% pulp and moderately associated with treatments N 96, 50% pulp and NT 116, 40% pulp while treatments N 96, 60% pulp, N 96, 40% pulp and VIN 13, 50% pulp were negatively associated with the attribute yeasty. The aroma attribute musty was moderately associated with treatments NT 116, 60% pulp, NT 116, 40% pulp and N 96, 50% pulp while the treatment VIN 13, 60% pulp was negatively associated with the attribute musty. The aroma attribute fruity was strongly associated with the treatment

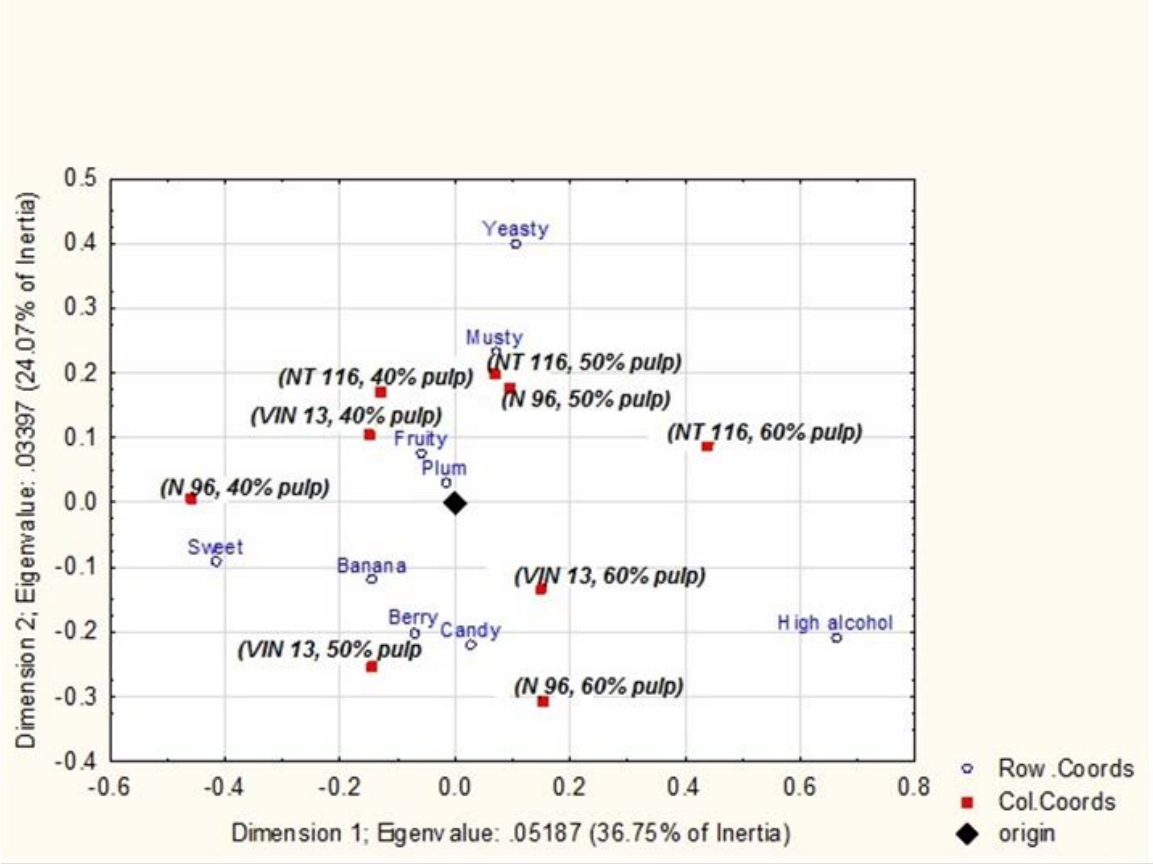


**Figure 4.2** CA plot of the uninstructed sorting task displaying the ***aroma*** sensory descriptors in relation to specific samples of alcoholic fermented **red-fleshed plum** beverages

N 96, 40% pulp and moderately associated with the treatment NT 116, 40% pulp. The aroma attribute plum was moderately associated with the treatment N 96, 40% pulp while the treatment VIN 13, 50% pulp was negatively associated with the attribute plum. The sweet aroma was strongly associated with N 96, 40% pulp and moderately associated with NT 116, 40% pulp while treatments NT 116, 50% pulp and N 96, 50% pulp were negatively associated with sweet as an aroma attribute. The aroma attribute banana was strongly associated with the treatment VIN 13, 50% pulp and moderately associated with the treatment N 96, 40% pulp, while the treatment NT 116, 40% pulp was negatively associated with the attribute banana. The aroma attribute berry was strongly associated with the treatment VIN 13, 50% pulp. The aroma attribute candy was strongly associated with the treatment N 96, 60% pulp while treatments N 96, 40% pulp and NT 116, 60% pulp were negatively associated with the attribute candy. The aroma attribute high alcohol was strongly associated with treatments NT 116, 60% pulp, N 96, 60% pulp and VIN 13, 60% pulp while treatments VIN 13, 40% pulp and NT 116, 40% pulp were negatively associated with the attribute high alcohol.

The CA plot for flavour (Figure 4.3) shows that the attribute berry was strongly associated with treatments NT 116, 60% pulp and VIN 13, 50% pulp and moderately associated with the treatment VIN 13, 60% pulp, while the treatment N 96, 60% pulp was negatively associated with the attribute berry. The sweet taste was moderately associated with treatments N 96, 40% pulp and NT 116, 60% pulp. The flavour attribute fruity was strongly associated with the treatment N 96, 50% pulp and moderately associated with the treatment VIN 13, 50% pulp while the treatment VIN 13, 40% pulp was negatively associated with the attribute fruity. The astringent mouthfeel was moderately associated with treatments VIN 13, 50% pulp and N 96, 60% pulp. The flavour attribute plum was strongly associated with treatments VIN 13, 40% pulp and NT 116, 50% pulp and moderately associated with treatments N 96, 40% pulp and NT 116, 40% pulp, while treatments VIN 13, 50% pulp, VIN 13, 60% pulp, N 96, 60% pulp and NT 116, 60% pulp were negatively associated with the attribute plum. The sour taste was strongly associated with treatments VIN 13, 40% pulp and NT 116, 40% pulp and moderately associated with the treatment N 96, 40% pulp, while treatments VIN 13, 50% pulp and NT 116, 60% pulp were negatively associated with the sour taste. The flavour attribute yeasty was strongly associated with the treatment VIN 13, 50% pulp and moderately associated with the treatment

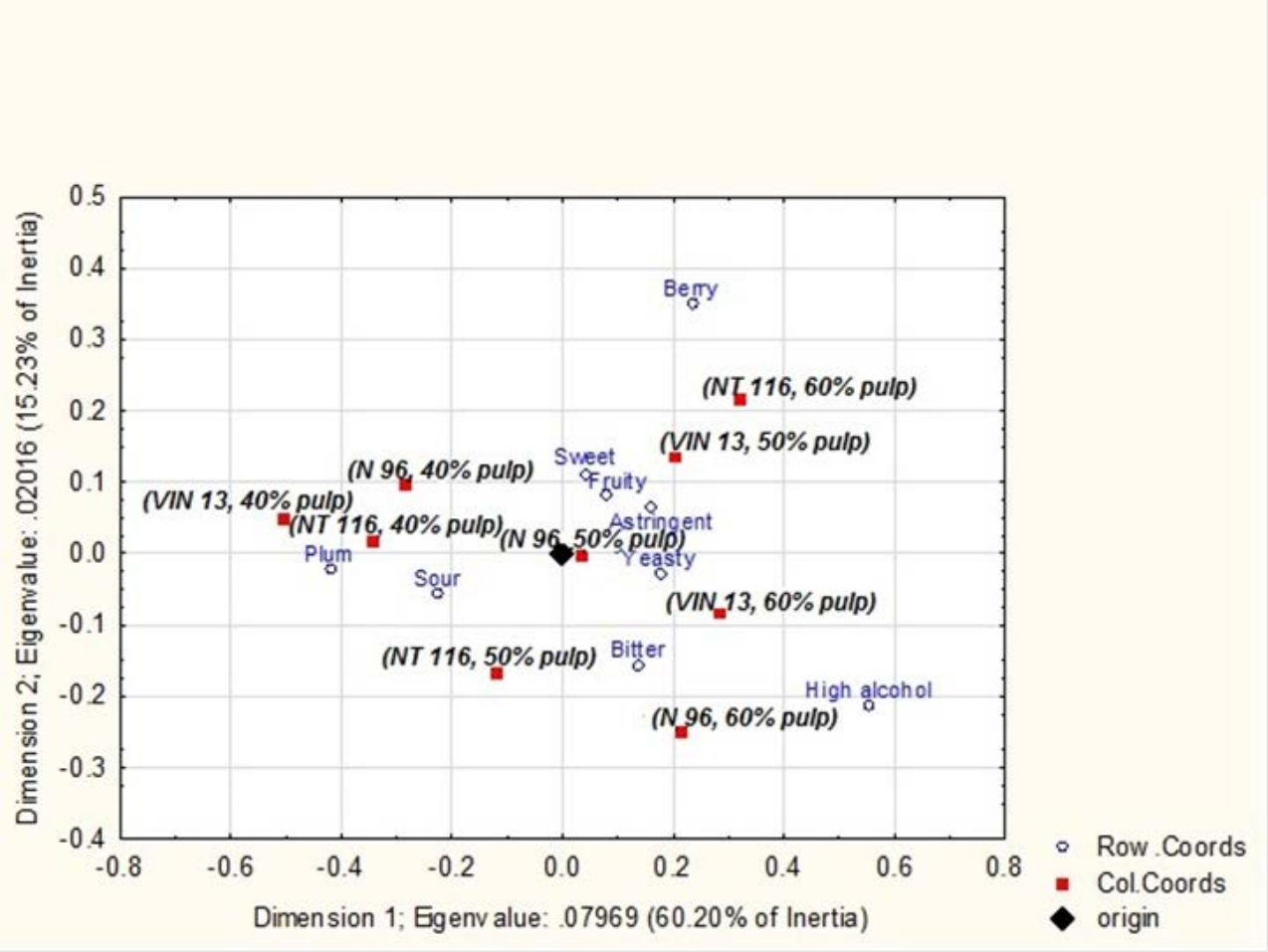


**Figure 4.3** CA plot of the uninstructed sorting task displaying the ***flavour*** sensory descriptors in relation to specific samples of alcoholic fermented **red-fleshed plum** beverages

VIN 13, 60% pulp. The bitter taste was strongly associated with the treatment N 96, 60% pulp and moderately associated with the treatment VIN 13, 60% pulp, while treatments N 96, 40% pulp and N 96, 60% pulp were negatively associated with the bitter taste. The flavour attribute high alcohol was strongly associated with treatments VIN 13, 60% pulp, N 96, 60% pulp and NT 116, 60% pulp and moderately associated with the treatment N 96, 50% pulp while treatments VIN 13, 40% pulp and NT 116, 40% pulp were negatively associated with the attribute high alcohol.

#### *4.6.4.3 Evaluating the sensory response for aroma and flavour for blackberry beverages using DISTATIS and CA plots*

The DISTATIS plot for aroma (Figure 4.4) shows that the sensory panel sorted the samples into three distinct groups. Samples in group 1 represented treatments NT 116, 60% pulp, N 96, 60% pulp and VIN 13, 60% pulp. Samples in group 2 represented treatments NT 116, 50% pulp, N 96, 50% pulp and VIN 13, 50% pulp, while samples in group 3 represented treatments NT 116, 40% pulp, N 96, 40% pulp and VIN 13, 40% pulp. Since three isolated groups were formed by the sensory panel, it indicated a high consensus level and the sensory panels' ability to classify the different beverage treatments. Therefore, the resulting groups sorted confirmed that the sensory panel were able to sort the treatments according to different aroma attributes. Hence, the results of DISTATIS plot (Figure 4.4) correlated with the results of the ANOVA for the chemical profile of blackberry confirmed that the independent variable yeast strain (VIN 13, N 96 and NT 116) did not have a significant ($p > 0.05$) effect.

The CA plot for aroma (Figure 4.5) shows that the attribute high alcohol was strongly associated with treatments VIN 13, 60% pulp, N 96, 60% pulp and NT 116, 60% pulp, while treatments VIN 13, 40% pulp, VIN 13 50% pulp, N 96, 50% pulp and NT 116, 40% pulp were negatively associated with the attribute high alcohol. The aroma attribute fruity was strongly associated with treatments N 96, 40% pulp and NT 116, 40% pulp and moderately associated with the treatment VIN 13, 40% pulp, while treatments VIN 13, 50% pulp, N 96, 50% pulp and NT 116, 50% pulp were negatively associated with the attribute fruity. The aroma attribute muscadel was strongly associated with treatments VIN 13, 40% pulp and N 96, 40% pulp and moderately associated with the treatment NT 116, 40% pulp, while treatments N 96, 50% pulp and NT 116, 50% pulp were negatively associated with the attribute

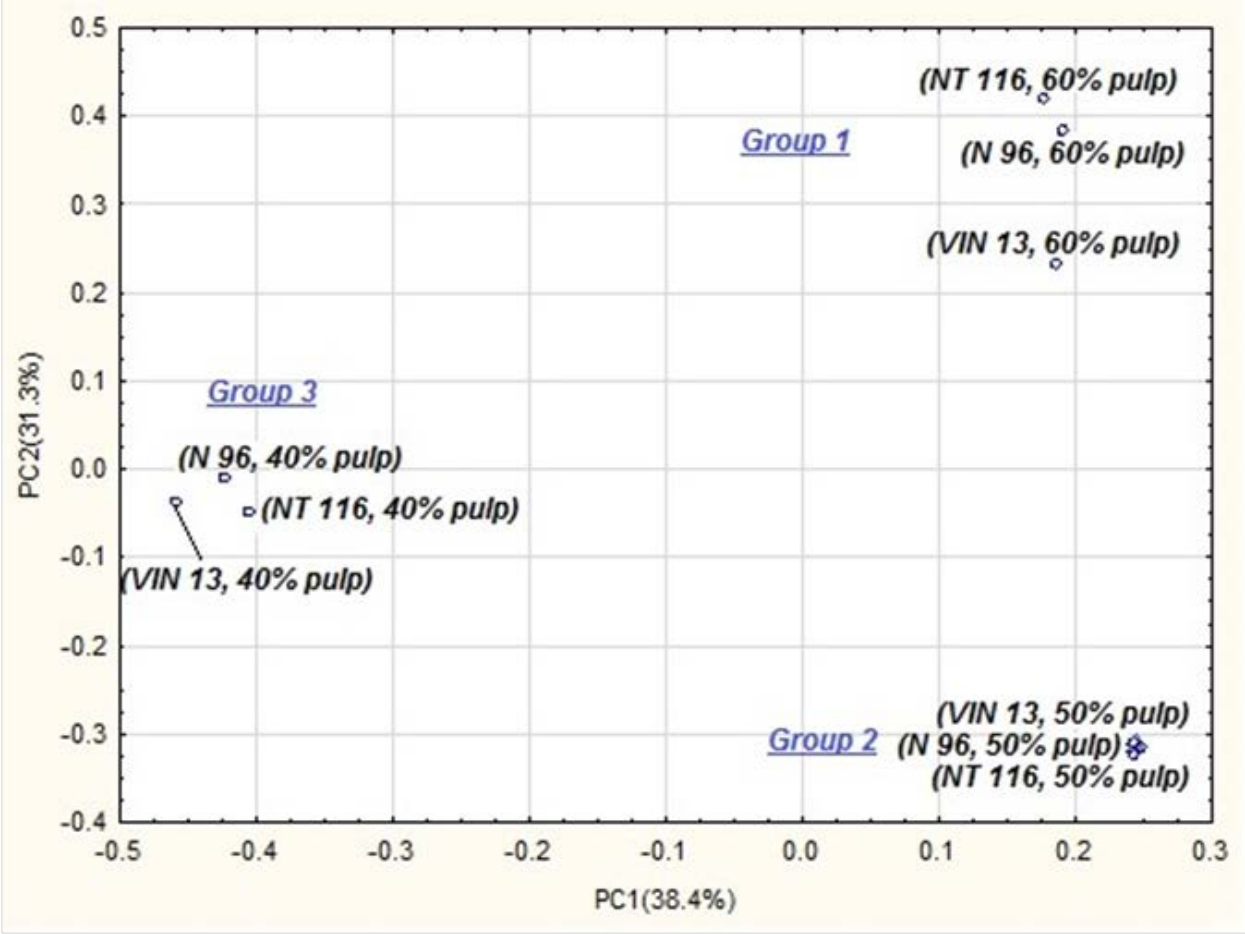


**Figure 4.4** DISTATIS plot, calculated from individual similarity matrices, representing the ***aroma*** similarities of **blackberry** beverages as perceived by the sensory panel during uninstructed sorting

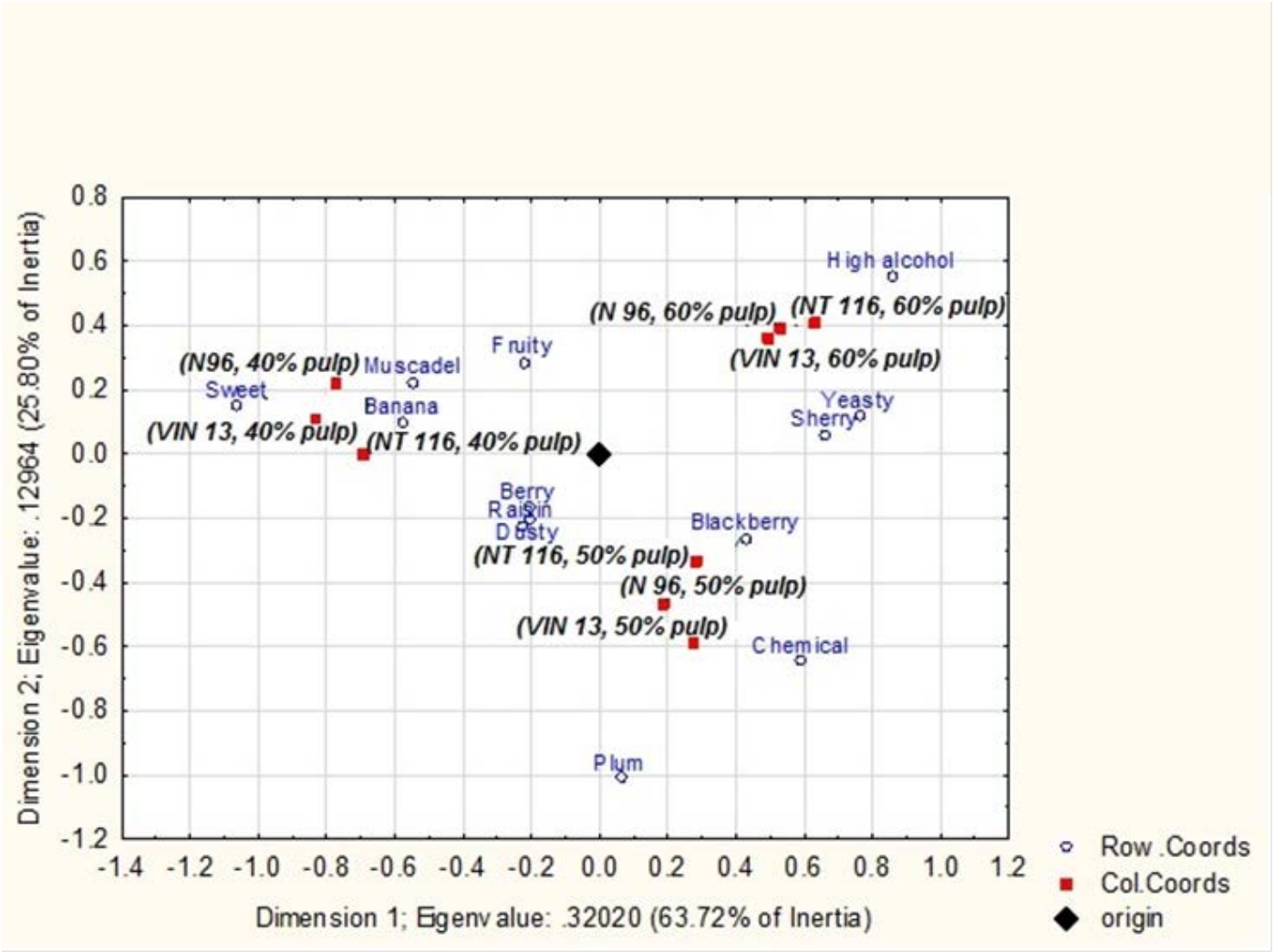


**Figure 4.5** CA plot of the uninstructed sorting task displaying the ***aroma*** sensory descriptors in relation to specific samples of alcoholic fermented blackberry beverages

muscadel. The sweet aroma was strongly associated with treatments VIN 13, 40% pulp, N 96, 40% pulp and NT 116, 40% pulp, while treatments VIN 13, 50% pulp, VIN 13, 60% pulp, N 96, 50% pulp, NT 116, 50% pulp and NT 116, 60% pulp were negatively associated with sweet aroma as an attribute. The aroma attribute yeasty was strongly associated with treatments VIN 13, 60% pulp, N 96, 60% pulp, NT 116, the treatment N 96, 60% pulp, while treatments VIN 13, 40% pulp and N 96, 40% pulp were negatively associated with the attribute blackberry. The aroma attribute chemical was strongly associated with treatments VIN 13, 50% pulp and N 96, 50% pulp, while treatments VIN 13, 40% pulp, N 96, 40% pulp and NT 116, 40% pulp were negatively associated with the attribute chemical. The aroma attribute plum was strongly associated with treatments VIN 13, 50% pulp, N 96, 50% pulp and NT 116, 50% pulp, while treatments VIN 13, 60% pulp, N 96, 40% pulp, N 96, 60% pulp and NT 116, 60% pulp negatively associated with the attribute plum.

The DISTATIS plot for flavour (Figure 4.6) shows that the sensory panel sorted the samples into three distinct groups. Samples in group 1 represented treatments NT 116, 60% pulp, N 96, 60% pulp and VIN 13, 60% pulp. Samples in group 2 represented treatments NT 116, 50% pulp, N 96, 50% pulp and VIN 13, 50% pulp while samples in group 3 represented treatments NT 116, 40% pulp, N 96, 40% pulp and VIN 13, 40% pulp. As was the case with the aroma attributes for these samples, three isolated groups were formed by the sensory panel, confirming sensory panels' ability to classify the different beverage treatments for these samples. Therefore, the resulting groups sorted confirmed that the sensory panel were able to sort the treatments according to different aroma attributes. Hence, the results of DISTATIS plot (Figure 4.6) correlated with the results of the ANOVA for the chemical profile of the blackberry (Table 4.5), confirming that the independent variable yeast strain (VIN 13, N 96 and NT 116) did not have a significant ($p > 0.05$) effect.

The CA plot (Figure 4.7) shows that the flavour attribute plum was strongly associated with treatments VIN 13, 40% pulp, VIN 13, 50% pulp, N 96 50% pulp, NT 116, 50% pulp, while treatments VIN 13, 60% pulp, N 96, 60% pulp and NT 116, 60% pulp were negatively associated with the attribute plum. The flavour attribute muscadel was strongly associated with treatments NT 116, 50% pulp and NT 116, 40% pulp and moderately associated with the treatment N 96, 50% pulp, while treatments N 96, 60% pulp and NT 116, 60% pulp were negatively associated with

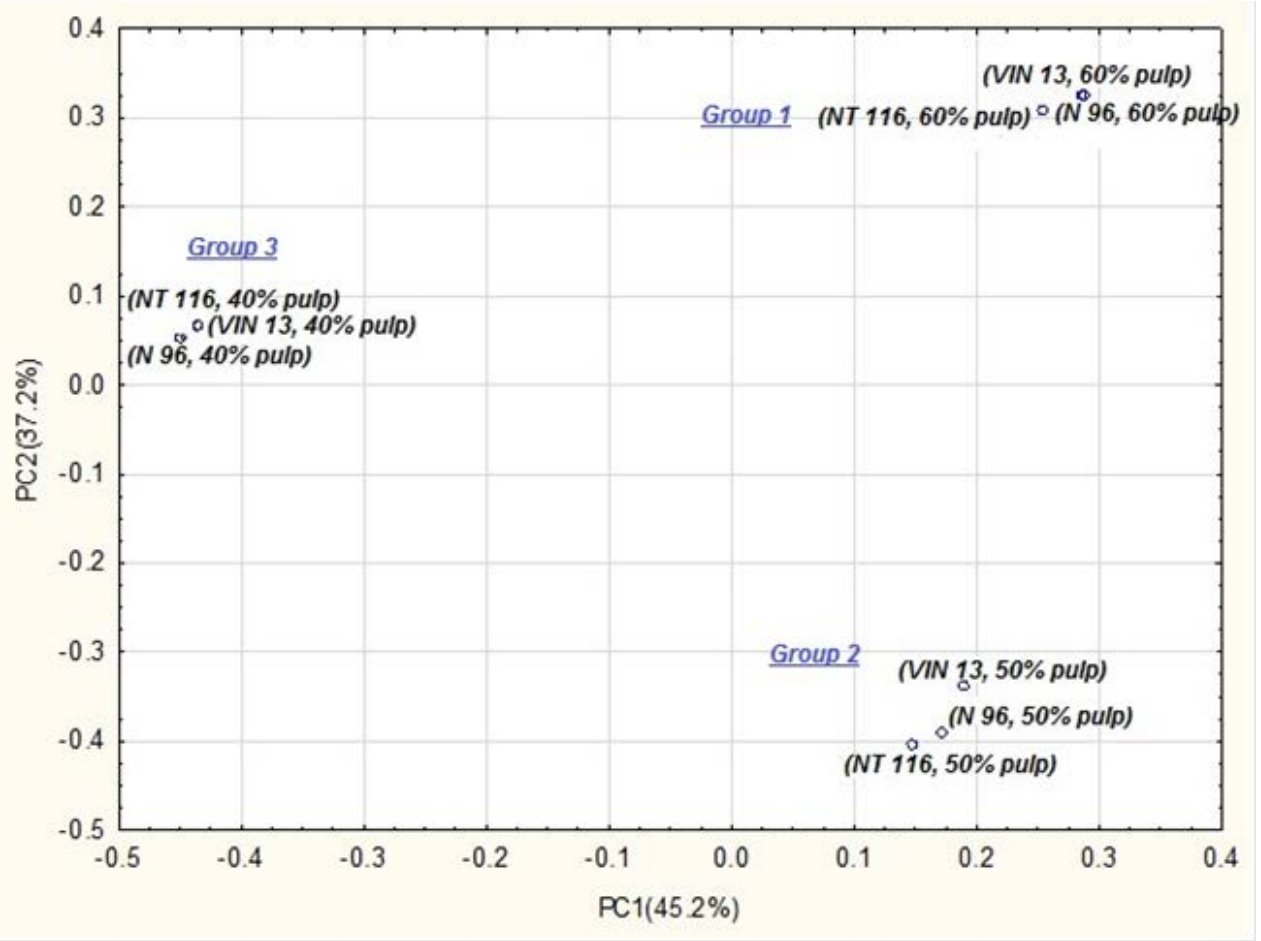


**Figure 4.6** DISTATIS plot, calculated from individual similarity matrices, representing the ***flavour*** similarities of **blackberry** beverages as perceived by the sensory panel during uninstructed sorting

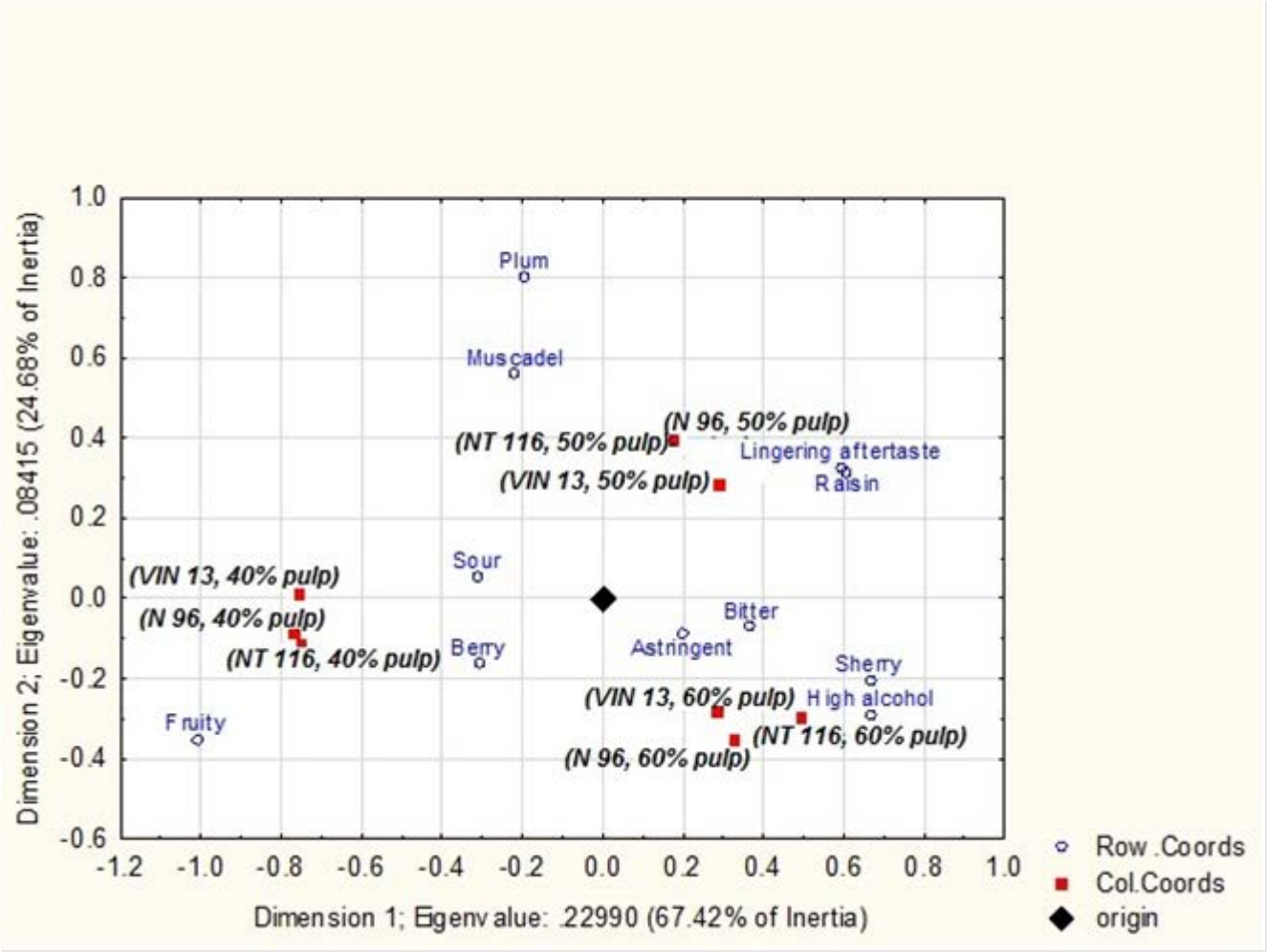


**Figure 4.7** CA plot of the uninstructed sorting task displaying the ***flavour*** sensory descriptors in relation to specific samples of alcoholic fermented **blueberry** beverages

the attribute muscadel. The lingering aftertaste was strongly associated with treatments VIN 13, 50% pulp and NT 116, 50% pulp, while treatments VIN 13, 40% pulp, N 96, 40% pulp and NT 116, 40% pulp were negatively associated with the attribute lingering aftertaste. The flavour attribute raisin was strongly associated with treatments N 96, 50% pulp, NT 116, 50% pulp and NT 116, 60% pulp and moderately associated with the treatment VIN 13, 50% pulp, while treatments VIN 13, 40% pulp, N 96, 40% pulp and NT 116, 40% pulp were negatively associated with the attribute raisin. The sour taste was strongly associated with treatments VIN 13, 40% pulp, N 96, 40% pulp and NT 116, 40% pulp, while treatments VIN 13, 50% pulp, N 96, 60% pulp and NT 116, 60% pulp were negatively associated with sour taste as an attribute. The bitter taste was strongly associated with treatments N 96, 60% pulp and NT 116, 60% pulp and moderately associated with the treatment N 96, 50% pulp, while treatments VIN 13, 40% pulp and N 96, 40% pulp were negatively associated with the attribute bitter taste. The astringent mouthfeel was strongly associated with the treatment NT 116, 60% pulp and moderately associated with the treatment VIN 13, 60% pulp, while the treatment NT 116, 40% pulp was negatively associated with the attribute astringent mouthfeel. The flavour attribute berry was strongly associated with treatments VIN 13, 40% pulp, N 96, 40% pulp and NT 116, 40% pulp, while treatments VIN 13, 50% pulp and NT 116, 50% pulp were negatively associated with the attribute berry. The flavour attribute sherry was strongly associated with treatments N 96, 60% pulp and NT 116, 60% pulp, while treatments VIN 13, 40% pulp, N 96, 40% pulp and NT 116 40% pulp were negatively associated with the attribute sherry. The flavour attribute high alcohol was strongly associated with treatments VIN 13, 60% pulp, N 96, 60% pulp and NT 116, 60% pulp, while treatments VIN 13, 40% pulp, N 96, 40% pulp and NT 116, 40% pulp were negatively associated with the attribute high alcohol. The flavour attribute fruity was strongly associated with treatments VIN 13, 40% pulp, N 96, 40% pulp and NT 116, 40% pulp, while treatments VIN 13, 50% pulp, N 96, 50% pulp, NT 116, 50% pulp and NT 116, 60% pulp were negatively associated with the attribute fruity.

#### *4.6.4.4 Evaluating the sensory response for **aroma and flavour** for blueberry beverages using DISTATIS and CA plots*

The DISTATIS plot for aroma (Figure 4.8) shows that the sensory panel sorted the samples into three distinct groups. Samples in group 1 represented treatments NT

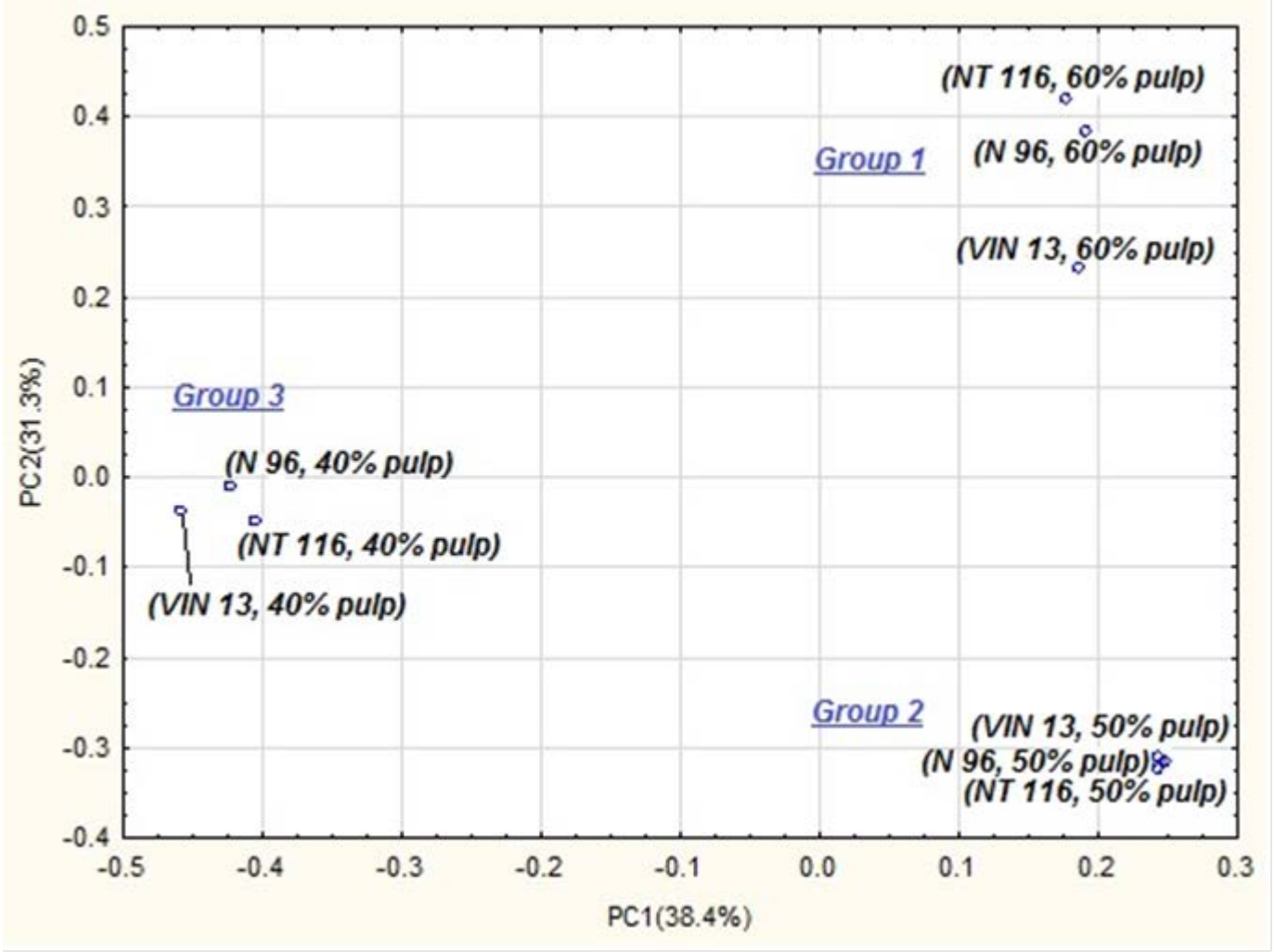


**Figure 4.8** DISTATIS plot, calculated from individual similarity matrices, representing the ***aroma*** similarities of **blueberry** beverages as perceived by the sensory panel during uninstructed sorting

116, 60% pulp, N 96, 60% pulp and VIN 13, 60% pulp. Samples in group 2 represented treatments NT 116, 50% pulp, N 96, 50% pulp and VIN 13, 50% pulp, while samples in group 3 represented treatments NT 116, 40% pulp, N 96, 40% pulp and VIN 13, 40% pulp. Since three isolated groups were formed by the sensory panel, it indicated a high consensus level and the sensory panels' ability to classify the different beverage treatments. Therefore, the results confirmed that the sensory panel were able to sort the treatments according to different aroma attributes. Moreover, the DISTATIS plot (Figure 4.8) shows grouping based on %pulp and not on yeast strain. This correlated with the results of the ANOVA for the chemical profile of blueberry (Table 4.6), confirming that the independent variable yeast strain (VIN 13, N 96 and NT 116) did not have a significant ($p > 0.05$) effect.

The CA plot for aroma (Figure 4.9) shows that the attribute green grass was strongly associated with treatments N 96, 50% pulp and NT 116, 50% pulp, while treatments VIN 13, 60% pulp, N 96, 60% pulp and NT 116, 60% pulp were negatively associated with the attribute green grass. The aroma attribute chemical was strongly was associated with treatments N 96, 50% pulp and N 96, 60% pulp, while treatments VIN 13, 40% pulp, VIN 13, 50% pulp and NT 116, 40% pulp were negatively associated with the attribute chemical. The aroma attribute banana was strongly associated with the treatment NT 116, 40% pulp, while the treatment N 96, 60% pulp was negatively associated with the attribute banana. The aroma attribute fruity showed no associations with any of the treatments since this aroma attribute was positioned close to the origin. The aroma attribute high alcohol was strongly associated with treatments VIN 13, 50% pulp, N 96, 60% pulp and NT 116, 60% pulp, while treatments VIN 13, 40% pulp, N 96, 40% pulp and NT 116, 40% pulp were negatively associated with the attribute high alcohol. The sweet aroma was strongly associated with treatments VIN 13, 40% pulp, N 96, 40% pulp and NT 116, 40% pulp, while treatments VIN 13, 50% pulp, N 96, 50% pulp, N 96, 60% pulp and NT 116, 60% pulp were negatively associated with sweet aroma as an attribute. The aroma attribute berry was strongly associated with treatments VIN 13, 40% pulp and NT 116, 40% pulp, while the treatment VIN 13, 50% pulp was negatively associated with the attribute berry. The aroma attribute musty was strongly associated with treatments N 96, 60% pulp and NT 116, 60% pulp, while treatments VIN 13, 40% pulp, N 96, 40% pulp and NT 116, 40% pulp were negatively associated with the

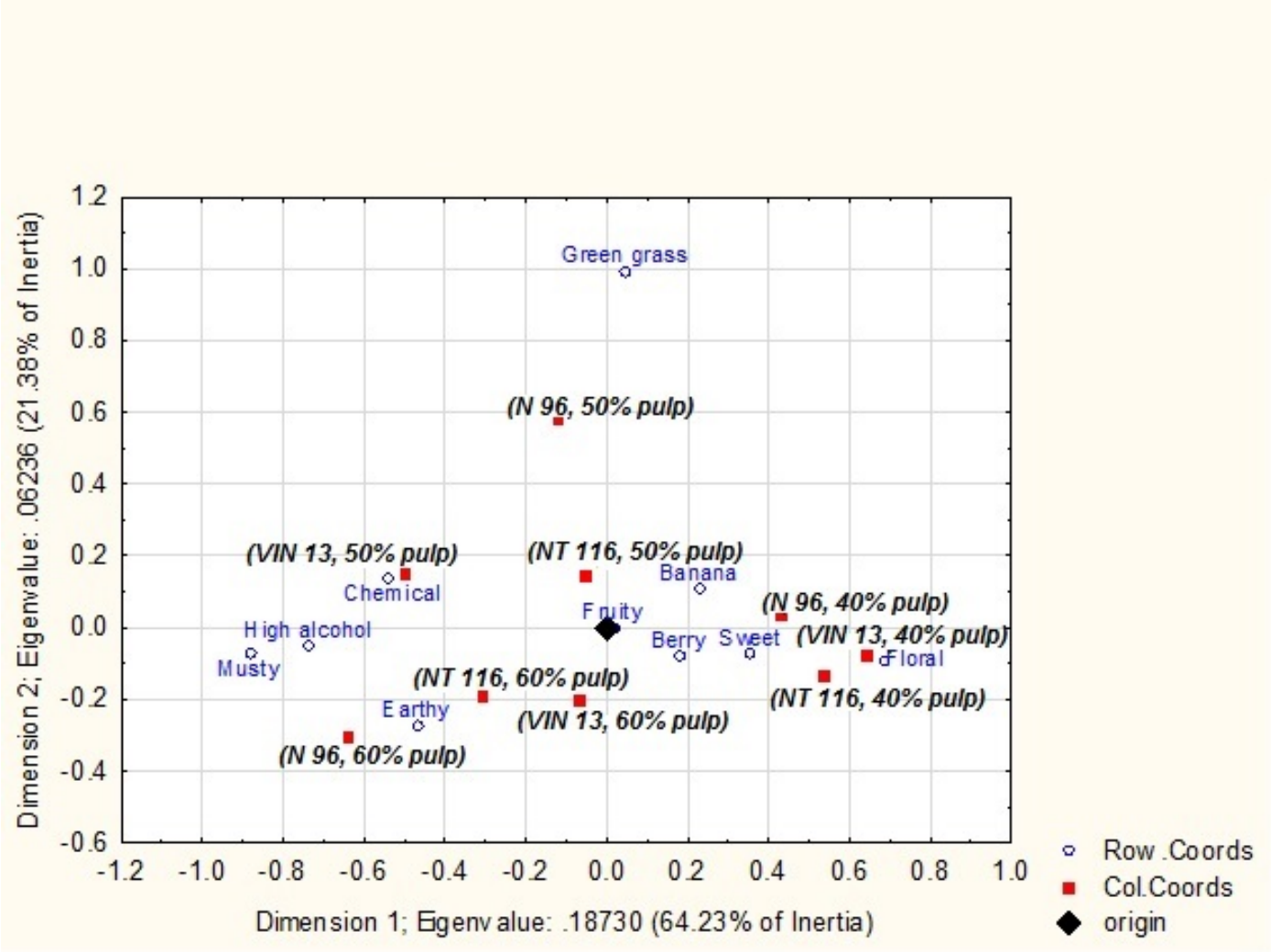


**Figure 4.9** CA plot of the uninstructed sorting task displaying the ***aroma*** sensory descriptors in relation to specific samples of alcoholic fermented **blueberry** beverages

attribute musty. The aroma attribute floral was strongly associated with treatments VIN 13, 40% pulp, 96, 40% pulp and NT 116, 40% pulp, while treatments VIN 13, 50% pulp, N 96, 60% pulp and NT 116, 50% pulp were negatively associated with the attribute floral. The aroma attribute earthy was strongly associated with treatments N 96, 60% pulp and NT 116, 50% pulp, while treatments VIN 13, 40% pulp and N 96, 50% pulp were negatively associated with the attribute earthy.

As was the case with the aroma attributes, the DISTATIS plot for flavour (Figure 4.10) shows that the sensory panel sorted the samples into three distinct groups. The grouping of the treatments was the same as for aroma, with the exception of VIN 13, 50% pulp, which grouped with the 60% pulp treatments. As was the case with the aroma for these samples, the results confirmed the ability of the sensory panel to differentiate between these samples based on the response to the different treatments. Hence, the results of DISTATIS plot (Figure 4.10) correlated with the results of the ANOVA for the chemical profile of blueberry (Table 4.6): albeit that two 50% samples grouped separately, one grouped with 60% pulp treatments, again confirming that the independent variable yeast strain (VIN 13, N 96 and NT 116) did not have a significant ($p > 0.05$) effect.

The CA plot for flavour (Figure 4.11) shows that the attribute floral was strongly associated with treatments VIN 13, 40% pulp, N 96, 40% pulp and NT 116, 40% pulp, while treatments N 96, 50% pulp and NT 116, 50% pulp were negatively associated with the attribute floral. The flavour attribute earthy was strongly associated with the treatment NT 116, 40% pulp. The astringent mouthfeel was moderately associated with the treatment N 96, 60% pulp. The bitter taste was strongly associated with treatments VIN 13, 50% pulp, N 96, 60% pulp and NT 116, 60% pulp, while treatments VIN 13, 40% pulp and NT 116, 40% pulp were negatively associated with bitter taste as an attribute. The flavour attribute high alcohol was strongly associated with treatments VIN 13, 50% pulp, N 96, 60% pulp and NT 116, 60% pulp, while treatments VIN 13, 40% pulp, N 96, 40% pulp and NT 116, 40% pulp were negatively associated with the attribute high alcohol. The sweet taste was moderately associated with treatments VIN 13, 50% pulp and NT 116, 60% pulp. The flavour attribute berry was strongly associated with strongly associated with the treatment VIN 13, 60% pulp. The flavour attribute yeasty was moderately associated with treatments VIN 13, 60% pulp and NT 116, 40% pulp. The sour taste was

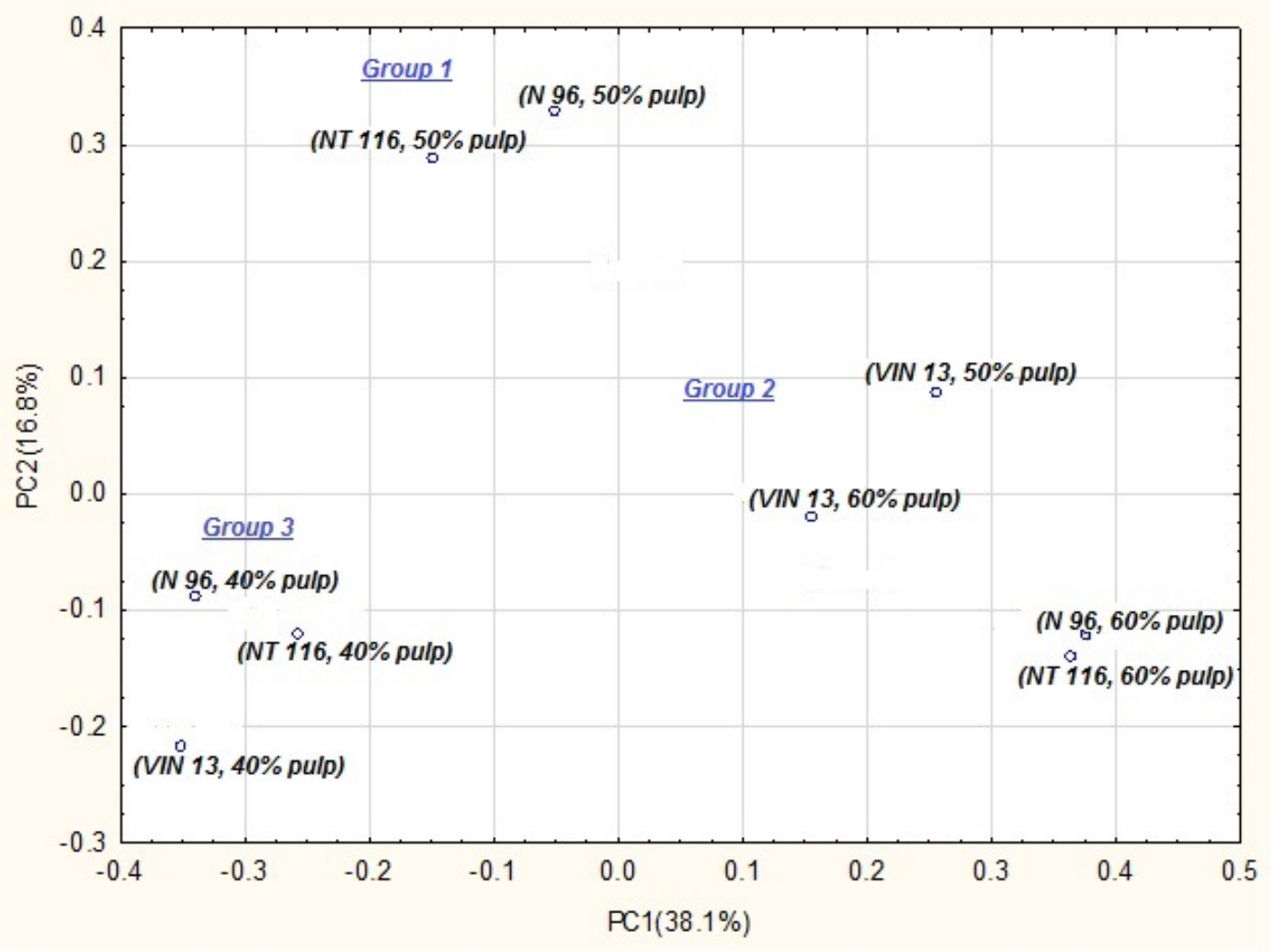


**Figure 4.10** DISTATIS plot, calculated from individual similarity matrices, representing the ***flavour*** similarities of **blueberry** beverages as perceived by the sensory panel during uninstructed sorting

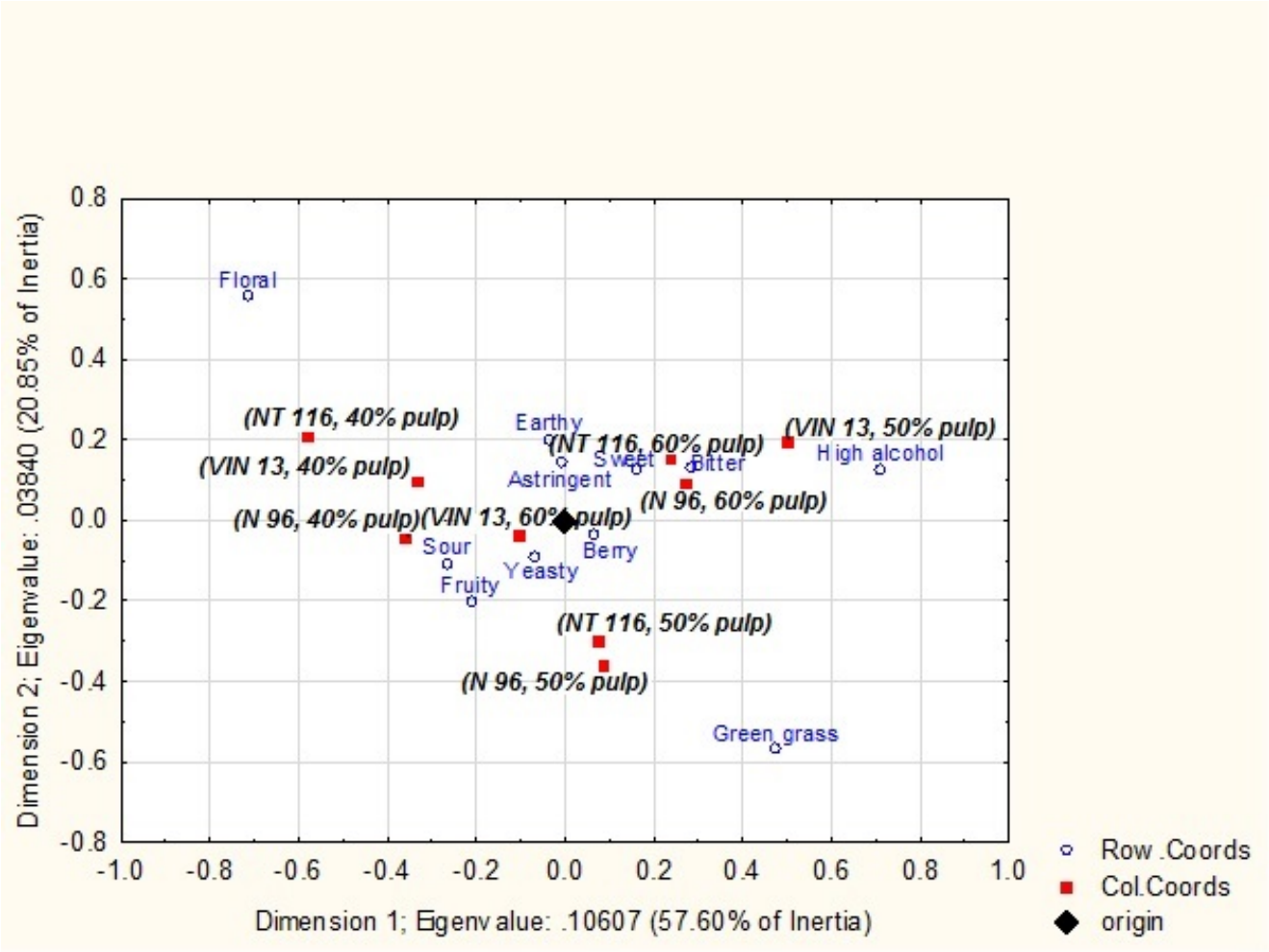


**Figure 4.11** CA plot of the uninstructed sorting task displaying the ***flavour*** sensory descriptors in relation to specific samples of alcoholic fermented **blueberry** beverage

strongly associated with treatments VIN 13, 40% pulp and N 96, 40% pulp and moderately associated with the treatment NT 116, 50% pulp, while treatments VIN 13, 50% pulp, N 96, 60% pulp and NT 116, 60% pulp were negatively associated with the sour taste as an attribute. The flavour attribute fruity was moderately associated with treatments VIN 13, 60% pulp and N 96, 50% pulp, while the treatment VIN 13, 50% pulp was negatively associated with the attribute fruity. The flavour attribute green grass was strongly associated with treatments VIN 13, 50% pulp, N 96, 50% pulp and NT 116, 50% pulp, while treatments VIN 13, 40% pulp, VIN 13, 60% pulp and NT 116, 40% pulp were negatively associated with the attribute green grass.

Overall, the results of the uninstructed sorting task for the alcoholic fermented fruit beverages (red-fleshed plum, blackberry and blueberry) confirmed that the sensory panel were able to use the uninstructed sorting technique to classify the alcoholic fermented fruit beverages in terms of aroma and flavour profiling. Moreover, for the blueberry and blackberry samples, the sensory groupings agreed with the results for routine chemical parameters, namely that %pulp and not yeast strain affected the response variables significantly ($p < 0.05$).

## 4.7 Consumer acceptability of alcoholic fermented red-fleshed plum, blackberry and blueberry beverages

The mean hedonic ratings are shown for the overall consumer response for all alcoholic fermented fruit beverage treatments developed in this study (Figure 4.12).

The mean rating for alcoholic fermented red-fleshed plum beverages showed that sample treatment N 96, 60% pulp had the highest rating at 7.96 which indicated that consumers rated these samples as “like very much”, while the remaining sample treatments showed a rating well above 6.7, i.e. consumers rated these sample treatments as “like moderately” with the exception of sample treatment VIN 13, 60% pulp which had a rating of 4.96. In other words consumers rated these samples as “neither like nor dislike”. The alcoholic fermented blackberry beverages showed hedonic mean ratings ranging from 3.12 – 4.28 (Figure 4.12), i.e. consumers rated these beverage as “dislike slightly”. In the case of alcoholic fermented blackberry beverages, the hedonic mean rating for alcoholic fermented blueberry beverages ranged from 4.04 – 4.92 (Figure 4.12). Hence, the blueberry beverages were similar to blackberry beverages, but slightly higher, but still resulting in a consumer response of “disliked slightly”.

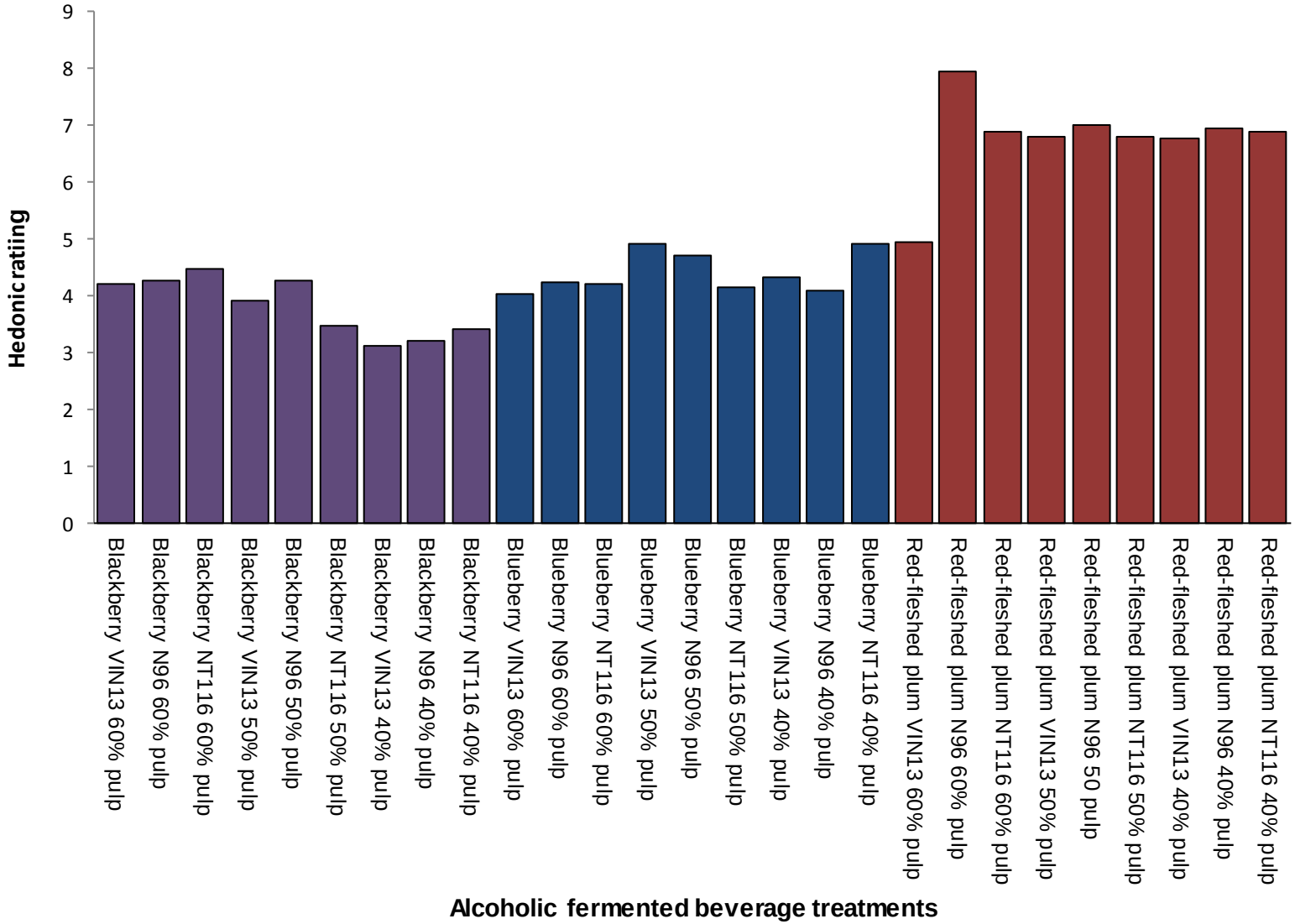


**Figure 4.12** Mean ± standard deviation consumer liking ratings (n = 25) for alcoholic fermented **red-fleshed plum, blackberry** and **blueberry** beverages

In terms of the overall consumer response for the alcoholic fermented fruit beverages (red-fleshed plum, blackberry and blueberry), alcoholic fermented red-fleshed plum beverages showed the highest consumer preference, while blackberry and blueberry showed the lowest consumer preference. The general acceptability of the blackberry and blueberry alcoholic fermented beverages in this case could potentially be influenced by the fact that the consumers have diverse food habits, attitudes, beliefs and opinions on food choice and purchase, which is particularly important in the acceptance or rejection of foods (Jaeger, 2006; Villegas *et al*., 2009). Hence, the fact that the red-fleshed alcoholic fermented beverage resembled red wine more closely in appearance and taste (results not shown), while the berry based beverages did not resemble any known wine could have resulted in the low consumer acceptability scores.

## 4.8 Conclusion

The study aimed to measure the dependent variables (DV) which constitute the key quality parameters for red wines (Du Toit & Lambrechts, 2002; Hayasaka *et al*., 2007; Walker & Blackmore, 2012; Lago-Vanzela *et al*., 2013), namely methanol, ethanol, titratable acidity, objective colour, total soluble solids, pH, sensory profile in response to two independent variables (ID), namely yeast strain and percentage pulp levels in order to adapt existing technologies towards producing an alcoholic fermented plum beverage based on red wine styles. From the results in this study it can be seen that the DV measured were similar to corresponding parameters of red wines. The different treatments affected the sensory profile of the alcoholic fermented fruit beverages (red-fleshed plum, blackberry and blueberry) which resulted in different sensory profiles for each of the abovementioned alcoholic fermented beverages. In terms of the overall consumer response, the alcoholic fermented red-fleshed plum beverages showed the highest preference amongst consumers, whereas alcoholic fermented blackberry and blueberry showed the lowest consumer preference. Hence, based on these parameters (ID and DV) applied in this study, it was demonstrated that it is possible to develop red wine styled alcoholic fermented beverages, using alternative fruit varieties (red-fleshed plums and selected berries) with acceptable overall consumer acceptability.

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# CHAPTER 5

# GENERAL DISCUSSION AND CONCLUSIONS

## 5.1 Discussion

Fruits are often used as the main source of the diversification in food formulations. (Müller *et al*., 2010). However, fresh fruit has limited shelf-life, causing product losses due to spoilage. Regulatory standards where pieces that do not fulfill the desired morphological requisites are not suitable for direct distribution compound the problem of product wastage (Gustavsson *et al*., 2011). In this context, the abundance and diversity of fruits produced in South Africa has great potential to be exploited in the food industry.

In countries where grapes are not in abundance, wine is produced from local fruits that are cheap and readily available. Moreover, although grapes are the main raw material used for wine production, there is an increasing interest in the search for other fruits, such as apricot, apple and palm sap, which are also appropriate for wine-making. In this context, the abundance and diversity of fruits produced in South Africa has great potential to be exploited in the food industry, for example in the production of fermented fruit beverages i.e. wine-style beverages (Dias *et al*., 2007; Duarte *et al*., 2009).

The methanol content is of major importance when producing fermented fruit beverages. The increase in methanol in fermented fruit beverages is often associated with activities of pectin methyl esterase (PME) and pectatelyase (PAL) enzymes present in fruit juices (Hou *et al*., 2008). The addition of commercial pectolytic enzymes (CPE) plays an important role in the process of making fermented fruit beverages, where it assists with extraction, clarification and filtration of fruit juice and wine puree to increase the yield and quality (e.g. pigment, flavour, clarity and viscosity) (Soufleros *et al*., 2002).

Therefore, utilization of ripe fruits or their juices for wine production is considered to be an attractive means of utilizing surplus and over-ripe fruits. From an agricultural viewpoint it is important to minimize post-harvest losses and considering the important role that agri-processing currently have on growing the agricultural

economic sector in South Africa and in particularly the Western Cape, extending the range of fermented fruit beverages will lead to creating employment and enhancing the diversity of the sector, thus creating a platform upon which to build a stable, more productive and growing sector (Anon, 2015).

The adaptation of existing technologies to develop high value products from underutilized agricultural produce, namely plums and selected berries, could be suitable for manufacturers who are not handling any primary agricultural produce to use these new technologies to produce fermented fruit products from commercially available juices or pulps. Thus, the industrial utilization of produce presently going to waste will be increased, highlighting the importance of utilizing agricultural produce by value-addition to contribute to future sustainability.

The aims of this study were (1) to measure methanol, ethanol, titratable acidity, objective colour, total soluble solids and sensory profile as a function of yeast strain and percentage pulp in order to adapt existing technologies toward producing new fermented fruit beverage products using plums, an under-utilized agricultural produce; and (2) to measure methanol, ethanol, titratable acidity, objective colour, total soluble solids and sensory profile as a function of yeast strain, pulp percentage and sugar levels in order to adapt existing technologies toward producing new fermented fruit beverages based on red and white wine styles, while applying the technology developed in the first part of the study using red-fleshed plums, blueberries and blackberries.

In these studies, the results showed that no methanol was detected and by adapting existing technologies toward producing alcoholic fermented fruit beverages utilizing plums and selected berries. The optimal combination of independent variables was ascertained, namely percentage pulp concentration (40%, 50% and 60% pulp) and yeast strain (VIN 13, N 96 and NT 116) in terms of the dependent variables, namely methanol, ethanol, titratable acidity, objective colour, total soluble solids, pH and sensory profile that constituted to the key quality parameters to formulate white and red wine style beverages that were sensorially acceptable (Lago-Vanzela *et al*., 2013; Sokolowsky *et al*., 2015).

In conclusion, the processing conditions developed and applied in these studies towards the development of alcoholic fermented beverages utilizing (plums and selected berries) demonstrated ways of improving the functionality of fruit commodities by developing niche products by implementing agro-processing. Hence,

the development of alcoholic fermented beverages utilizing (plums and selected berries) showed potential for micro agro-industries, as well as the impact on its fundamental role in employment creation and income generation.

## 5.2 Recommendations

It is recommended that further work needs to be done to chemically characterize the sensory data using HS-SPME and the 5975 Series GC/MSD system to successfully develop a profiling method as a means to understand the variables involved in alcoholic fermented fruit beverages acceptability (Coelho *et al*., 2015). As well look at the usefulness of intermediate products of plum processing for alcoholic fermentation and the chemical composition of plum distillates (Balcerek *et al*., 2013). During the processing and production of alcoholic fermented plum and berry beverages, at the end of the fermentation phase it is recommended that as an alternative to racking the clear fermented beverage in each bottle, centrifugation could be considered as a means to increase the yield and clarity of the alcoholic fruit beverages. Furthermore, regarding the liquor regulations in South Africa, regulations should be added towards classes of alcoholic fruit beverages made from fruit other than grapes, as well as alcohol percentage levels for these classes (Anon., 2012), thus, making provision for alcoholic fermented plum and selected berry beverages that were developed in these studies.

## 5.3 References

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