THE EFFECT OF ENZYMATIC PROCESSING ON BANANA JUICE AND WINE

By

George William Byarugaba-Bazirake

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Promoter: Prof Pierre van Rensburg
Co-promoter: Prof William Kyamuhangire

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DECLARATION

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SUMMARY

Although bananas are widely grown worldwide in many tropical and a few sub-tropical countries, banana beverages are still among the fruit beverages processed by use of rudimentary methods such as the use of feet or/and spear grass to extract juice. Because banana juice and beer remained on a home made basis, there is a research drive to come up with modern technologies to more effectively process bananas and to make acceptable banana juices and wines. One of the main hindrances in the production of highly desirable beverages is the pectinaceous nature of the banana fruit, which makes juice extraction and clarification very difficult.

Commercial enzyme applications seem to be the major way forward in solving processing problems in order to improve banana juice and wine quality. The particular pectinolytic enzymes that were selected for this study are Rapidase CB, Rapidase TF, Rapidase X-press and OE-Lallzyme. In addition this study, investigate the applicability of recombinant yeast strains with pectinolytic, xylanolytic, glucanolytic and amylolytic activities in degrading the banana polysaccharides (pectin, xylan, glucan starch) for juice and wine extraction and product clarification.
The overall objective of this research was to improve banana juice and wine by enzymatic processing techniques and to improve alcoholic fermentation and to produce limpid and shelf-stable products of clarified juice and wine. The focus was on applying the selected commercial enzyme preparations specifically for the production of better clarified banana juice and wine. This is because the turbid banana juice and beer, which contain suspended solids that are characterised by a very intense banana flavour, require a holistic approach to address challenges and opportunities in order to process pure banana beverages with desirable organoleptic qualities.

The specific objectives of applying commercial enzymes in the processing of banana juice and wine, comparing with grape winemaking practices, use of recombinant yeast and analyses of various parameters in the juices and wines made have enabled generation of information that could be of help to prospective banana juice and wine processors.
The research findings obtained could be used to establish a pilot plant or small-scale industry in the banana processing beverages producing large quantities,and finally the overall objective of obtaining limpid and shelf stable products would be achieved.

OPSOMMING

Hoewel piesangs wêreldwyd in ‘n verskeidenheid tropiese en enkele subtropiese lande gekweek word, bly piesangdrankies onder die minderwaardige tropiese vrugtesappe en -wyne, hoofsaaklik as gevolg van ‘n gebrek aan waardetoevoeging. Hierdie waardetoevoeging kan as “lank agterstallig” beskryf word op grond van die onbekombaarheid van hierdie produkte in die mark, hoewel dit ook ‘n noodsaaklike vertraging kan wees op grond van die problematiese aard van die verwerking van piesangvrugte na kwaliteit drankies. Een van die vernaamste hindernisse in die produksie van hoogs aanloklike drankies is die pektienagtige aard van piesangvrugte, wat sapekstraksie en -verheldering in die proses van drankvervaardiging baie bemoeilik.

Kommersiële ensiempreparate blyk die vernaamste roete te wees om verwerkingsprobleme op te los om sodoende die kwaliteit van piesangsap en -wyn te verbeter. In hierdie studie het ondersoeke die toepasbaarheid toegelig van pektinolitiese, xilanolitiese, glukanolitiese en amilolitiese aktiwiteite in die afbreking van piesangpektien en -stysel om sap- en wynekstraksie en -verheldering te vergemaklik. Die spesifieke pektinolitiese ensieme wat vir hierdie studie gekies was, is Rapidase CB, Rapidase TF, Rapidase X-press en OE-Lallzyme. Hierdie kommersiële ensiempreparate het ‘n noemenswaardige rol gespeel. Kommersiële proteases was bruikbaar vir waasstabilisering.

Die oorhoofse doelwit van hierdie navorsing was om plaaslike piesangsap en -wyn deur middel van ensimatiese verwerkingstegnieke te verbeter en om die alkoholiese gisting daarvan na waardetoegevoegde, helder en rakstabiele produkte bestaande uit verhelderde sap en wyn te verbeter. Die fokus was op die toepassing van geselekteerde kommersiële ensiempreparate spesifiek vir die produksie van piesangsap en -wyn wat beter verhelder is. Die rede hiervoor is dat troebel piesangsap en -bier met ‘n groot hoeveelheid gesuspendeerde vaste stowwe en ‘n baie intense piesanggeur steeds op plaaslike en internasionale markte as minderwaardig beskou word en dus waardetoevoeging deur die vermindering van gesuspendeerde vaste stowwe en piesanggeur benodig.

Die spesifieke doelwitte van die toepassing van kommersiële ensieme tydens die verwerking van piesangsap en -wyn en die ensimatiese effek op ander wesenlike

parameters is tydens hierdie studie bereik. Die navorsingsbevindings wat verkry is, kan ‘n loodsprojek of ‘n kleinskaalse bedryf in die piesangverwerkingsektor van stapel stuur en uiteindelik die oorhoofse doelwit van ‘n verbetering in piesangsap en -wyn vir kommersiële doeleindes bereik.

DEDICATION

This dissertation is dedicated to those whose efforts have culminated in this work: my late parents for their contribution to my upbringing and also to my late brother, Constante, whose constant (sometimes forceful) encouragement ensured that I attended school and ultimately obtain this degree.

BIOGRAPHY

George William Byarugaba-Bazirake was born in Kabale district, Uganda, East Africa on 3 April1960. He passed Primary Leaving Examination in 1st Grade 1975, obtained the East African Certificate of Education (EACE) and Uganda Advanced Certificate of Education (UACE) commonly known as Higher School Certificate (HSC) in 1979 and 1981 respectively at Makobore High School, Kinyasaano. He trained at the National Teachers College Kakoba, Mbarara as a Grade Five Teacher and graduated in 1984.

George enrolled at Kuban State University of Technology, Krasnodor in the former Soviet Union (Russia) in 1990 and obtained an MSc degree in Sugar Technology in 1995. He enrolled at Stellenbosch University for a PhD in Wine Biotechnology in the year 2002.

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PREFACE

This dissertation is presented as a compilation of six chapters. Each chapter has been introduced separately**.**

Chapter 1: GENERAL INTRODUCTION

Chapter 2: LITERATURE REVIEW

Fruit juices and wines and factors that affect their production

Chapter 3: RESEARCH RESULTS

Characteristics of enzyme treated banana juice from three cultivars of tropical and sub-tropical Africa.

Chapter 4: RESEARCH RESULTS

Influence of commercial enzymes on banana wine extraction and clarification and their effects on sensory properties.

Chapter 5: RESEARCH RESULTS

Characterisation of banana wine extracted and clarified with aid of recombinant (DNA) yeast.

Chapter 6: GENERAL CONCLUSION AND RECOMMENDATIONS

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CHAPTER 1
GENERAL INTRODUCTION

1.1 BACKGROUND

Bananas belong to the family Musaceae and genus *Musa*. *Musa* spp. already provided man with food, tools and shelter prior to recorded history. Bananas are major crops of West and East Africa and are grown in some 120 countries throughout the developing world (see Appendices 2 and 3).

World banana production, according to available statistics, was 80.6 million tons per annum in the early 1990s (Food and Agriculture Organization, 1994), with Africa producing about 30 million tons per annum (Food and Agriculture Organization, 1996). According to the latest FAO statistical records as reported by the International Institute of Tropical Agriculture (IITA, 2003), more than 58 million tons of bananas and 30 million tons of plantains were produced worldwide in 2000. India is the largest banana producer with an output of 16 million tons per annum and Uganda ranks second to it producing 12 million tons per annum (Sunday Monitor, 2007). South Africa produces 300 000 tons of bananas per annum (De Beer, 2004).

Banana is the fourth most important crop after rice, wheat and maize and international trade in bananas is valued at around US$5 billion per annum (Sunday Monitor, 2007). Traditional banana juice extraction and its subsequent fermentation to produce beer (*tonto*) is an important social and economic activity among many tribes in East Africa (Stover and Simmonds, 1987; Davies, 1993). Likimani (1991) reported that *tonto* is a popular traditional beverage in Burundi, Uganda and Rwanda. Banana juice and beer may however contain suspended solids which are not desired by some consumers. Therefore, efforts are made to reduce suspended solids by applying different processing technologies such as the addition of enzymes to pulps.

Although bananas have been traditionally dietary staples in many countries in Tropical Africa, they have until recently been relatively neglected by most policy makers and research institutions partly due to high post-harvest losses coupled with difficulty in marketing and processing of these highly perishable commodities

(Olorunda, 2000). Generally, the most recent estimates of losses of cooking bananas and plantain differ in different countries and were 0-10% for Kampala in Uganda (Digger, 1994) and as high as 35% for the Ivory Coast (N'Guessan, 1991).

Biotechnology and other related technologies such as genetic engineering have played a significant role in food processing technologies. Enzymes play a pivotal role in the winemaking process. In addition to enzymes that occur in pre-and post fermentation operations, there are many more different enzymes driving the fermentation kinetics that convert grape juice to wine. Commercial enzyme preparations are widely used as supplements since the endogenous enzymes from yeasts and other micro organisms present in must and wine are often neither efficient nor sufficient under winemaking conditions to efficiently catalyse the various biotransformation reactions (for a detailed review on enzymes in winemaking, see Van Rensburg and Pretorius, 2000). Pectolytic enzyme preparations have been used with great success for many years in the fields of food technology (Ough and Berg, 1974). In wine and fruit juices, these enzyme preparations are mainly used to yield more juice and increase the press capacity (Wörner and *et al*., 1998). The use of pectolytic enzyme preparations has been reported to affect sensory quality, since these preparations often also contain other enzyme activities (for example, cinnamylesterase, glucosidase, oxidase) that can have a negative effect on wine (Lao *et al*., 1997). The best wines are produced when the desired enzymatic activities are optimally reinforced and the negative effects restricted to a minimal level (Van Rensburg and Pretorius, 2000).

In this study, commercial enzymes were applied and we report their effects on juice yield, clarification and organoleptic properties of banana juice and wine from three banana cultivars.

1.2 STATEMENT OF THE PROBLEM

Uganda ranks number two after India in banana production, but ranks seventienth country worldwide in terms of economic benefits from bananas. Many communities produce banana products of a low quality, particularly banana juice and beer. These banana beverages are not being exported to regional or international markets. One of the primary reasons seems to be a lack of processing technologies as required to

improve the quality of the beverages. Secondly, the methods of juice extraction are cumbersome and require significant energy expenditure in juice extraction operations and thus such methods may not be efficient for large scale production.

This research attempted to solve the problems encountered in the production of banana beverages through improved methods of juice extraction, filtration and clarification by applying commercial enzyme preparations. The approach intended to ease juice extraction, improving yields and clarification (limpidity), as well as creating haze-free beverage for long shelf-life.

1.3 OBJECTIVES OF THE STUDY

The overall objective of this study was to improve the quantity and quality of banana juice and wine by enzymatic processing of banana pulp and improved alcoholic fermentations aiming at limpid and shelf stable products.

The specific objectives of this study were to:

1. apply commercial enzymes to extract and clarify banana juice and wine, and evaluate enzyme effects on the organoleptic and other properties;
2. analyse and compare any changes in relevant parameters such as sugar and alcohol content, VA, TA, reducing sugars, pH, turbidity, etc., in the inoculated fermentations;
3. apply winemaking practices used for grape wine production and assess if there are any similarities in wine character and stability of banana wine;
4. use recombinant (DNA) yeast strains transformed with pectinase, glucanase, amylase and xylanase to inoculate banana pulp, extract wine and analyse physicochemical characteristics of resulting wine.

1.4 SIGNIFICANCE AND IMPACT OF THE STUDY

The commercial enzyme preparations used in the study are suitable for the production of banana juice and wine, and seem to be better alternatives to the traditional methods, which use mainly manual methods such as spear grass (*Imperata cylindrica*) for juice extraction. The enzymatic clarification of banana juice

and wine could lead to medium-scale or even large scale industrial banana beverages production in areas where the banana raw material is in abundance.

1.5 SCOPE OF THE STUDY

The research was limited to enzymatic processing of banana juice and wine from three cultivars (*Musa*, genotypes AAA, AAA-EA and ABB), i.e. ”Bogoya”, “Mbidde” and “Kayinja” respectively. A comparative study in terms of physicochemical characteristics was done on one cultivar (*Musa,* AAA genotype) known as *Williams* in sub-tropical South Africa and as *Gros Michel* in tropical Uganda. The enzymes that were used were selected based on their capability to influence higher juice yields than the others after carrying out preliminary experiments with various enzymes. The enzymes used in the study were pectinases, xylanases, glucanases, amylases and proteases.

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CHAPTER 2
LITERATURE REVIEW: FRUIT JUICES AND WINES AND FACTORS THAT AFFECT THEIR PROCESSING

2.1 INTRODUCTION

In this chapter juices and wines made from various fruits are discussed. As a guideline to this research project, common parameters and methods were looked at.

2.2 INDIGENOUS FRUIT JUICES AND WINES – THE TRADITIONAL APPROACH OF PROCESSING

The traditional way of processing fruit juices involves rupturing fruits by mechanical means for juice extraction, yielding cloudy raw juices and eliminating the waste. The extracted juice can be clarified by several clarification treatments, which yield a clear juice product that may be concentrated or not (Pilnik, 1996). Winemaking involves mainly three categories of operations, *viz:* pre-fermentation, fermentation and post-fermentation operations (Iland *et al*., 2000; Jackson, 2000; Ribéreau-Gayon *et al*., 2000).

In the case of wines made from grapes, pre-fermentation involves crushing the fruit and releasing juice. In case of white wine, juice is separated from the skin whereas in red wine the skins are not separated from the juice. Clarification of juice for white wine is usually achieved by sedimetation or centrifugation. Then yeast is added to the clarified juice to initiate fermentation. In red winemaking, the pulp, skins and seeds of grapes are kept together after crushing and during all or part of the fermentation. This is done to extract colour and flavour. Yeast is added to mashed pulp (must) in red winemaking.

Fermentation involves a reaction that converts the sugars in the juice into alcohol and carbon dioxide. Yeasts utilise the sugars during the yeast fermentation period. A stuck fermentation occurs when yeasts do not completely utilise the available sugar and the fermentation rate slows down and/or ceases. Clarification may be achieved by racking, filtration and/or centrifugation. Fermentation proceeds under anaerobic conditions and may be boosted with diammonium phosphate (DAP) to supplement nitrogen required for yeast growth in non-traditional approach of winemaking. Post-

fermentation practices are done after fermentation has reached the desired stage or when fermentation is complete. Here, wine is racked off the yeast lees, usually in stainless steel vessels or in oak barrels. During the storage period, the wine may be filtered, cold stabilised, fined and/or blended. Various fining agents such as enzymes, bentonite, diatomaceous earth, egg albumen etc. may be commercially purchased and added to aid in clarification of wines. Wine undergoes continued changes during maturation and at an appropriate stage, the wine is filtered and bottled.

While wines made from grape present a well-established and to such advanced economic activity, the extraction and subsequent fermentation of banana juice into banana beer is an important social and economic activity among many tribes in East Africa (Munyanganizi-Bikoro, 1975; Stover and Simmonds, 1987; Davies, 1993). The brewing of banana beer is not only popular in Uganda, but also in Tanzania, Rwanda and the Democratic Republic of Congo (Davies, 1993). Some of the problems associated with banana juice processing include the high viscosity of the pulp, causing difficulties with juice extraction (Dupaigne and Dalnic, 1965; Viquez, *et al*., 1981), and browning problems (Galeazzi and Sgarbieri, 1981; Mao, 1974).

The traditional approach in the production of banana beer involves a number of steps. The ripe peeled and unpeeled beer bananas are mashed to extract the juice. The juice is usually diluted with water to attain a reduced soluble solids content of 12°Brix. Unmalted brown seeded sorghum (*Sorghum bicolor*), which is previously roasted and coarsely ground, is mixed with the diluted juice. The mixture is approximately made at a rate of 1 kg of sorghum flour to 10L of juice. The mixture is normally fermented in a wooden canoe-shaped container and covered well with banana leaves, spear grass (*Imperata cylindrica*) and spent banana pulp (pomace). The mixture is allowed to ferment spontaneously (with no yeast added) and this fermentation process takes two days on average. The brew is then siphoned off and stored in 20 L jerry cans at room temperature, and is ready for consumption. Packaging is done by siphoning out the brew from the particles and sealing it tightly in containers. The quality and alcohol content of the banana beer depend on the degree of dilution of the original juice, the amount and quality of sorghum adjuncts used and the conditions of alcohol fermentation. Usually, well-flavoured beer with a bitter taste, brown-golden colour and low alcohol content (2 to 5% v/v) is produced

from diluted juices (for details on banana beer processing, see Kyamuhangire and Pehrson, 1999; Gensi *et al*., 2000). Strong banana beer with an alcohol content of 11 - 15% is produced from undiluted juice (Davies, 1993). This banana beer has an average shelf-life of about five days. In Uganda, excess beer is further processed into a spirit called *waragi* through a process of distillation (Aked and Kyamuhangire, 1996). The spirit is normally consumed in double or triple-distilled form after further purifying processes at industrial level.

2.2.1 Juices and wines from tropical and subtropical fruits

Many tropical and subtropical fruits, including grapes, apples, pears, apricots, berries, peaches, cherries, oranges, mangoes, bananas and pineapples yield good amounts of juice on extraction. Upon fermentation, fruit juices can be changed into wines. However, the premium raw material for winemaking has been the grape, although attempts to process other fruit wines are being made. The techniques used for the production of other fruit wines closely resemble those for the production of wines made from white and red grapes. The differences arise from two facts. It is somewhat more difficult to extract the sugar and other soluble materials from the pulp of some fruits than it is from grapes, and secondly the juices obtained from most of the fruits are lower in sugar content and higher in acids than is true for grapes (Amerine *et al*., 1980).

As a solution to the above mentioned problems, the use of specialised equipment to thoroughly chop or disintegrate the fruits such as berries, followed by pressing to extract juice from the finely divided pulp, solves the first problem. The second problem is solved by the addition of water to dilute the excess acid and the addition of sugar to correct the sugar deficiency (Amerine *et al*., 1980).

The most frequently used non-grape fruit sources for the production of wines include apples, pears, plums, cherries, currants, oranges and various types of berries, etc. We will now focus on the research findings of a few fruit juices that have been processed into wines. Some of the juices extracted for winemaking were obtained by enzyme-treatment of the fruit pulps and those served as references in the investigations of this study.

Apple cider and wine

Apple (*Malus domestica*) fruit is used to prepare mild alcoholic beverages which are more nutritious than distilled liquors (Bhutani *et al*., 1989; Gasteineau *et al*., 1970; Joshi and Thakur, 1994). The apple fruit is more associated with cider than any other alcoholic beverages (Amerine *et al*., 1967; Joshi 1995; Sandhu and Joshi, 1994). Cider is a low alcoholic drink produced by fermentation of apple juice and is believed to have been produced for over 2000 years. Cider is known by different names around the world such as *cidre* (France), *sidre* (Italy), *sidra* (Spain) and *apfel wein* (Germany and Switzerland). Cider can be sweet or dry. Depending upon the alcohol content, cider is categorised into soft cider (1-5%) or hard cider (6-7%) (Downing, 1989; Joshi, 1995). Sparkling ciders contains low sugar levels and CO_2, usually sweet cider and still cider contain no CO_2 while dry cider contains little sugar and an alcohol content of about 6-7% (Joshi *et al*., 2000). The optimum temperature for cider fermentation ranges from 15 to18^{0}C. Sulphur dioxide provides a clean fermentation and prevents enzymatic browning of the juice (Beach, 1957) besides the control of microorganisms in the must (Amerine *et al*., 1967) and produce a cider of consistent quality (Poll, 1993). Simultaneous fermentation of apple juice with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* produced a cider with acceptable level of alcohol and acidity (O'Reilly and Scot, 1993). Mostly stainless steel tanks are used these days for fermentation of cider (Downing, 1989) though traditionally barrels of oak were used for this purpose. A temperature of 4^{0}C is suitable for bulk storage of ciders. After fermentation, the cider is racked and filtered. During aging, most of the suspended material settles down leaving the rest of the liquid clear which may be clarified with bentonite, casein or gelatin followed by filtration. After aging and clarification ciders needs to be pasteurized at 60^{0}C for about 20-30 minutes or SO_2 can be used (Joshi *et al*., 2000).

Apple wine is another product made from apple juice by alcoholic fermentation and has alcohol content of 11 - 14%. Like cider, apple juice or concentrate is the basic raw material, but as the alcohol content of wine is more than that of cider, amelioration with sugar or juice concentrate is essential (Joshi *et al*., 2000). Addition of ammonium salts to fermenting solution reduces the higher alcohol production in wine due to non-degradation of amino acids of the must (Reazin *et al*., 1970). Washing and crushing of the fruits, adding 50 ppm of SO_2 and 10% water in the making of apple wine is recommended (Vogt, 1977). Addition of diammonium hydrogen phosphate improved the fermentability (Joshi and Sandhu, 1996).

Palm Sap wine

In many parts of Africa and Asia, the sap obtained from various species of palm (*Acrocomia mexicana*) is fermented to produce wine called palm wine, coyol wine, Vino de coyol or toddy (Dahlgren, 1944; Dransfield, 1976). The wine is essentially a heavy milky white, opalescent suspension of live yeast and bacteria with a sweet taste and vigorous effervescence (Okafor, 1975 a;1975 b).

In the preparation of wine, the sap is obtained from decapitated inflorescence. A method for palm sap wine production was described by Balick (1990). The sap is collected in earthenware's which contain the yeasts and bacteria and even left-over toddy from the previous batch. The fermentation starts as soon as the sap flows into the pot. The freshly harvested sap is sweet in taste. The palm sap is a colourless liquid containing 10-12% sugar (Okafor, 1975 b). Further analysis revealed that the juice contains 4.20±1.4% sucrose, 3.31±0.95% glucose and 0.38±0.15% NH_3. Based on the vitamin B_{12} content, the occurrence of bacteria related to *Zymomones mobilis* was reported in fresh palm juice (Van Pee and Swings, 1971).

The sap is allowed to ferment spontaneously for about 24 hours before the product is soled. The wine bottles have constant foaming / bubbling due to the fermentation process called *boiling wine* or 'herviendo'. To continue fermenting, sugar has to be added to the palm juice. Normally, in 30 kg of sap, 4.5 kg of sugar is added per day and in a few days the wine must be sold, otherwise it gets converted into vinegar. During the fermentation process, the LAB lowers the initial pH of the juice from 7.4 to 6.8 and after 48hour, the pH is further reduced as low as 4.0. The ethanol content usually does not rise above 7.0% (Bassir, 1967) although ethanol levels as high as 12.86% was reported (Balick, 1990). Measuring changes in the ethanol content during fermentation revealed that after 24 hours the wine contained 1.5 to 2% and after 72 hours the ethanol content was at 4.5 to 5.2%. After 24 hours of fermentation, the organic acids present were measured as follow: lactic acid (32.1-56.7 mg/100ml), acetic acid (18.6-28.6 mg/100ml) and tartaric acid (11.7-36.0 mg/100ml). Coyol or palm sap wine is a source of minerals especially potassium (Balick, 1990). The physicochemical characteristics of a typical coyol wine as analysed by Balick (1990) were as follows: pH (4.0), alcohol (12.86%), and in ppm the minerals analysed were: phosphorus (38), sodium (28), calcium (142), magnesium (57), iron (2.5), manganese (0.5), copper (0.9), zinc (0.2) and potassium (2,540). The protein content of the wine was about 0.61%.

Coconut Toddy

An alcoholic beverage known as *toddy* is obtained by natural (spontaneous) fermentation of coconut palm inflorescence sap (Nathanael, 1955). The sap is traditionally collected in clay pots, sterilised by inverting over a flame for 5 minutes, and allowed to ferment in open pots for up to 2 days. During the period of sap collection microorganisms from the atmosphere enter the clay pots and multiply in the palm sap. The sap contains15-18% sucrose, which after fermentation results in the formation of a product containing about 7% (v/v) ethanol. Freshly prepared toddy has an average alcohol content of 7.9% (v/v), TA of 14.36 mM HCl per 100 ml, and pH of 3.7. Quality and yield problems of *toddy* are caused by uncontrolled spontaneous fermentation of sap (Nathanael, 1955).

Toddy is generally stored at ambient temperature. The storage temperature markedly affects its physico-chemical characteristics like pH, alcohol content, acidity and microbial count. In *toddy* stored at ambient temperature (27^0C), the alcohol content increased on the first day of storage, TA increased for up to 7 days, while microbial count decreased on the first and second days of storage. In *toddy* stored at 17^0C, the pH remained constant, but it dropped considerably when the *toddy* was stored at 37^0C (Faranndez *et al.,* 1980). Changes in *Tuba* (a popular fermented coconut sap in Phillipines) during the first week storage at various temperatures were recorded, and it was concluded that *Tuba* should not be sold more than 2 days after collection to maintain a high alcohol content and low titratable acidity (Faranndez *et al*., 1980).

Spontaneous fermentation of coconut palm by wild yeast was found to produce ethanol content much below the theoretical yield (Kalyananda *et al*., 1977). Such fermentation of coconut sap is brought about by a succession of heterogenous microorganisms consisting of yeast and bacteria (Atputharajah *et al*., 1986). Some microorganisms transform sugar in the sap into ethanol, while the others may merely survive or bring about other biochemical changes in the sap such as metabolism of ethanol. The alcohol content in toddy can be increased if growth of non-fermenting organisms is inhibited during fermentation (Liyanage *et al*., 1981). The presence of *Saccharomyces marxianus* along with *Saccharomyces exiguus* and *Candida* from the samples of fermented coconut palmrah palm wine (*toddy*) was reported (Liyanage *et al.,* 1981). From toddy fermentation, yeasts were isolated, grouped and characterised as maximum ethanol producers (9% ethanol), medium ethanol producers (4-6% ethanol) and trace or non-ethanol producers.

Saccharomyces chevlier, Schizosaccharomyces pombe, Pichia ohmeri and *Kloeckera javanica*; all produced about 9% ethanol on the 5th day of fermentation (Atputharajah *et al*., 1986).

Peach wine

The peach (*Prunus persica*) fruit can also be utilized for preparation of wine (Joshi and Bhutani, 1995; Shah and Joshi, 1998). The method of making wine from peach consists of dilution of the pulp in the ratio of 1:1 with water, raising the initial TSS to 24^{0}Brix and adding pectinol at the rate of 0.5%. Usually, 100 ppm of SO_2 is added to the must to control the undesirable microflora. Differences in fermentation behaviour of different peach cultivars and the composition of fruits of different cultivars were investigated (Joshi *et al*., 1997). Treatment of wines with wood chips of *Quercus* gave wine of best sensory qualities (Joshi and Shah, 1998; Joshi *et al*., 1999). Changes during maturation of wine included increases in tannins and esters, besides improvement in sensory qualities. Production of wine from dried peaches was not successful (Amerine *et al*., 1980; Pipet *et al*., 1977; Stanciulescu *et al*., 1975).

Apricot wine

Apricot (*Prunus ameniaca*) is a delicious fruit grown in many parts of hilly temperate countries. Wild apricot is used locally in the hills of Himachal Pradesh (India) to make liquor, though the method is very crude and reportedly is a result of natural fermentation, followed by distillation (Joshi,1995). A method for preparation of wine from wild apricot was developed which consists of diluting the pulp in the ratios of 1:2, addition of pectinol at the rate of 0.5% and fermentation using yeast *S. cerevisiae* (Joshi *et al*., 1990). Further, with the increase in the dilution level, the rate of fermentation, alcohol content and pH of the wines increased whereas a decrease in TA and VA, phenols, TSS, colour values and minerals (K, Na, Ca, Mg, Zn, Fe, Mn and Cu) took place.

In apricot wine from New Castle Variety, the extraction of pulp either by heat or addition of enzyme and water to the fruits could be adopted (Joshi and Sharma, 1994).

Dilution of apricot pulp with water in the ratio 1:1 and raising the TSS to 30^{0}Brix made wine of superior quality.

Jambal wine

Jambal fruit (*Synzygium cumini*) is liked for its refreshing pink to greyish flesh with a balanced sugar, acid and tannin contents and it is believed that the fruit has therapeutic value (Shrotri *et al*., 1963; Khurdiya and Roy, 1985). The fruit can be used in making dry wine of an acceptable quality (Shukla *et al*., 1991). Of the three cultivars investigated, Jamun made the best wine. To prepare Jambal wine the mash is usually diluted in a 1:1 ratio, and then the must is ameliorated to 23^0B with cane sugar, diammonium hydrogen phosphate is added at 0.2%, sulphur dioxide (normally up to 150 ppm,) and 0.25% pectinol enzyme. The fermentation is carried out with 2% *S. cerevisiae* followed by racking, filtration and bottling. The typical chemical characteristics of Jamun wine is normally as follows: alcohol (11.23%), TA (0.37g/100ml citric acid), VA (0.036% acetic acid) and pH 3.50.

Pear wine

In Europe, special varieties of pears with high tannin content are used for making “perry”, a fermented pear beverage (Amerine *et al*., 1980). For preparation of perry, the pears are grated and pressed in a rack and cloth press, after which the sugar and acidity levels are adjusted to suit the type of wine to be made. About 100 ppm of sulphur dioxide and wine yeast are added and the juice is left to ferment. The wine is racked, aged with oak chips, clarified and filtered. Pectinolytic enzymes are used to enhance the process of clarification (Amerine *et al*., 1980). The perry is preserved by pasteurisation. And depending upon the requirement, the wine can be sweetened, fortified or blended with other fruit wines. The production of perry can be a promising alternative for utilization of sand pear fruit, since sand pear have a very limited outlet for its direct consumption (Joshi, 1995). Initial trials on production of low alcohol beverage (perry) from sand pear were reported (Azad *et al*., 1986). Sand pear does not contain sufficient nitrogen for rapid fermentation and exogenous source of nitrogen has to be added. The procedure used for perry production includes raising the TSS to 20^0B, adding about 0.1% DAP and carrying out the alcoholic fermentation at 22 ± 1^0C by adding yeast culture at a rate of 5%. The effect of addition of different sugar sources (sucrose, glucose, honey, molasses and jaggery) on fermentation behaviour and sensory quality of perry was investigated (Azad *et al*., 1986). The highest rate of fermentation was recorded in must made with jaggery, followed by

honey, glucose, fructose, and molasses and lowest in case of sucrose. The sensory evaluation of perry of different treatments after 6 months of maturation showed the product made with jaggery had a more attractive colour than others, while in taste, all the products were comparable except that from molasses, which was unacceptable. Except for a little after-taste, the product made from jaggery had the best overall acceptability. A perry having 5% alcohol, TSS of 10^0B and a 0.5% acid content was found to be acceptable in sensory quality.

Cherry wine

Sour cherries (*Prunes*) are recommended in preference to sweet cherries for making wine (Schanderl and Koch, 1957). A blend of currant and table variety of cherries may be used to serve the purpose. To enhance the flavour, about 10% of the pits may be broken down while crushing the cherries. However, production of hydrogen cyanide from hydrolysis of amygdalin, present in the pit of cherries was reported (Baumann and Gierschner, 1974; Benk *et al*., 1976; Misselhorn and Adam, 1976; StadelMann, 1976). The cherry fruit has been found more suitable for preparation of dessert wine than table wine. The alcohol content of cherry wines may range from 12 to17%. To prepare a dessert wine of 16% alcohol, each litre of juice should be ameliorated with 430g of sugar. Addition of potassium metabisulphite is advisable before fermentation to play the role of antimicrobial agent and anti-oxidant. Use of pectic enzymes to improve clarification in cherry wine was recommended (Yang *et al*., 1950). The addition of urea to the cherry must did not improve the fermentability (Yang and Weighand, 1940). Cherry wine does not require a long aging period. Sugar may be added to sweeten the wine prior to bottling. The bottled wine can be preserved by either sterile filtration or pasteurization.

Orange Wine

Oranges (*Citrus sinensis*) are the base for a fortified, sweet dessert orange wine that is dark amber in colour. Research work on production of orange wine has been reviewed by Amerine *et al*. (1980). Only ripe sound fruit should be used for wine making. Juice, for fermentation, need to be extracted in a juice extractor (Kimball, 1991). Orange wines darken rapidly and develop a harsh, stale taste unless a fairly high level of sulphur dioxide is maintained. To avoid the stale flavour, the fruit must

not be overripe. Only the juice, without the peel, is extracted to avoid excessive oil from the peel, which slows fermentation. Wine preparation includes sweetening juice with 150g/L of sugar, the addition of potassium metabisulphite (100ppm), pectinol enzyme at 0.5% and DAP at the rate of 0.1% (Joshi, 1995). The wines are further sweetened by the addition of 2-3% sugar followed by pasteurisation and bottle maturation. Bitterness, a characteristic of citrus fruits is always associated with orange wine (Joshi and Thakur, 1994).

Mango Wine

Mango (*Mangifera indica*) contains proteinaceous substances, vitamins, minerals and is suitable for conversion into wine (Joshi, *et al*., 2000, Akingbala *et al*., 1992). Preliminary screening of ten varieties of mango for wine making was reported (Kulkami *et al*., 1980). For making wine, the fruits must first be pulped. The TSS is raised to 20^0B by adding cane sugar; usually 100 ppm SO_2 is used, pectinase enzyme (0.5%) is added to the pup. The must is fermented using *S. cerevisiae* at a rate of 10% for 7-10 days at 22^0C. After racking and filtration, the wine is treated with bentonite and bottled with 100ppm SO_2 as potassium metabisulphite. A sweet fortified wine, known as '*Dashehari*' is made by stopping the fermentation by adding 10% (v/v) mango brandy after 5 days of fermentation. For making sweet wine, cane sugar is added at the rate of 5g/L. The alcohol content of mango wines ranged from 5 to 13% and the wines normally contain low levels of tannins. Acceptable table wine was also prepared (Akingbala *et al*., 1992) from overripe mango fruit. The chemical properties of mango wine is normally as follows: pH of around 3.70, ash of 0.27g/100g, extract of 0.41g/100g, soluble solids of 5.0 Brix, specific gravity at 30^0C of 0.9812, TA of 0.38% (as citric acid), and 13.82% (v/v) ethanol.

Guava wine

The guava (*Psidium*) fruits which are available in abundance at low price have the potential to be utilised for production of highly acceptable fruit wine both for indigenous consumption and for export (Joshi *et al*., 2000). The fruit though has low sugar; it has a characteristic flavour and a golden yellow colour. Wine can be prepared either from guava juice or from guava pulp. For making wine from pulp, dilution with water is essential and a dilution rate of 1:2 was found to work better than

1:3. The treatment of pulp with pectinases increases the final yield of wine with about 18% (Bardiya *et al*., 1974). However, fermentation of guava pulp in the presence of pectinases reportedly yielded a wine with high tannin content, dark colour and an astringent taste. On the other hand, wine prepared from guava juice obtained by only treating the pulp with pectinases for juice extraction gave wine with a lower tannin content, optimum colour, flavour and an acceptable sensory quality. When the Brix reached 10^0B, the pomace is removed and more sugar is added (10%) to the fermenting materials and the mixture is allowed to ferment further (Bardiya *et al*., 1974). The better wine was obtained by fermentation of guava juice compared to the pulp.

Red raspberry wine

Raspberry (*Rubus idecus*) is also used for preparation of wine. Raspberry is prone to spoilage if not cooled promptly to 0^0C and can be preserved for 2-3 days (Joshi *et al*., 2000). Fermentation of pulp, depectinised juice and pasteurized juice affected the composition and other chemical characteristics of raspberry wine (Rommel *et al.* (1990). During fermentation, anthocyanin pigment is partially degraded with a total loss of at least 50% after storage. Cyanidin-3-glucoside was the most unstable anthocyanin, disappearing completely during fermentation while cyanidine 3-sorphoroside (the major anthocyanin) was the most stable pigment. It was concluded that pasteurized depectinized wine which has undergone fining had the most stable colour and best appearance after storage (Rommel *et al*., 1990). Some of the chemical characteristics of juice and wine is as follows: in juice a pH of 3.22, TA of 1.84%, TSS of 10.0^0Brix, total manomeric anthocyanin of 57.6, colour density of 16.4, percentage polymeric colour of 4.50 and haze of 3.60%; in wine a pH of 3.30, a TA of 1.73%, TSS around 05^0Brix total manomeric anthocyanin of 37.9, colour density of 9.60, percentage polymeric colour of 12.4 and a haze of 3.10% according to Rommel *et al*., (1990).

Strawberry wine

Strawberries (*Fragaria xananassa*) are used to prepare wine of good quality which has the appealing colour of a premium rose wine. But the attractive colour is many times short lived (Joshi *et al*., 2000). The juice is usually ameriolated to 22^0Brix by

addition of cane sugar. The must is mixed with 1% of ammonium phosphate and the fermentation is initiated by adding 1% yeast culture at a temperature of 16^0C. Fermentation of the strawberry juice continues until 0.1-0.2% reducing sugars are left over. After fermentation is completed the wine is racked, bottled and stored in the dark (Pilando *et al*., 1985). Ascorbic acid can accelerate the destruction of anthocyanin pigments and also contributes to browning (PoeiLangston and Wrolstad, 1981). Treatment with enzymes (mainly pectinases) inhibits polymerization and increases colour extraction and colour intensity in strawberry wine (Maurer, 1973; Flores and Heatherbell, 1984).

Grapefruit wine

Grapefruit may be used in the production of table wine, dessert wine and cordials. Like orange wines, the wines are somewhat bitter. The procedure of grapefuit winemaking is the same as that of orange winemaking. If the wine is too high in acidity, a calculated amount of potassium carbonate or calcium carbonate may be added and the mixture is heated to 65.6^0- 71.1ºC to hasten the reaction and to make the calcium citrate less soluble, afterwards filtered hot and then cooled down. Ion-exchange treatment may also be used to reduce acidity (for a detailed review on grapefruit wine, see Amerine *et al*., (1980).

Banana Beer and Wine

The technologies used in the traditional approach of processing banana beer in Uganda were based on indigenous knowledge such as the use of spear grass and feet to extract juice from bananas and subsequent addition of sorghum flour as an adjunct upon fermentation of the juice into a banana beer. Banana (*Musa peradisiaca*) fruits can also be converted into wine (Kundu *et al*., 1976). Bananas are peeled and homogenized in a blender for about 2-3 minutes to obtain a pulp. Potassium metabisulphite (100 ppm) can be added to prevent browning and to prevent growth of undesirable micro organisms. Fermentation is carried out at 18 ± 1^0C. Kotecha *et al*. (1994) carried out preliminary studies to optimize banana juice extraction by using different levels of pectinase enzymes and different incubation periods at 28 ± 2^0C. Based on these studies a 0.2% pectinase addition and a 4hr incubation time were selected for obtaining the juice from the pulp. The juice

was separated by centrifugation and the clear juice was used for preparation of wine (Kundu *et al*., 1976). The juice recovery from over-ripe bananas was higher (67.6%) than that from normal fruits (60.2%). Good quality wine was obtained from over-ripe banana fruit (Kotecha *et al*., 1994; Akingbala *et al*., 1992). The banana (*Musa peradisiaca*) wine chemical composition reported by Kotecha *et al*., (1994) was as follows: a TSS of 10.2±0.2, acidity of 0.88±0.06%, 3.18±0.16% reducing sugars, 0.044±0.002% tannins and alcohol of 6.06±0.06% (v/v). Whereas Akingbala *et al.* (1992) reported the chemical properties of a *Musa acuminata* wine as follows: ethanol 13.98% (v/v), TA of 0.33% (as citric acid), specific gravity at 30^0C of 0.9810, soluble solids as 5.2 Brix, an extract of 0.43g/100g and pH of 3.85.

Pineapple Wine

Pineapple (*Ananas comosus*) juice has sugar of up to 22-25^0Brix and can produce wine of about 12-13% alcohol, which can be preserved by pasteurisation (Joshi *et al*., 2000). Amerine *et al*. (1980) reported that the flavour of pineapple is not stable and oxidation can occur easily in the wine. The pineapple wine can be fortified and sweetened according to the desire of the consumer. Wine from pineapple waste is made in Hawaii and Philippines to make distilled vinegar.

Marula liqueur

Ripe Marula (*Sclerocarya birrea* sub. *caffra)* fruits are gathered, the kernels are removed in a destoner and the flesh is crushed from the skin. The marula flesh is then fermented under conditions similar to those used in winemaking. The thickness of the marula pulp results in problematic fermentations and can be diluted with water in a 1:1 ratio to reduce the viscosity of the juice (Fundira *et al*., 2002). After fermentation, the marula wine is distilled in copper pot-stills. The young liqueur is then matured in small oak casks for approximately two years and enriched with marula extract obtained through a special process that captures the unique flavours of the marula in a concentrated form. The spirit is then blended with fresh cream until a smooth consistency is reached. The creaming process is of a very high quality, resulting in a product that is stable, rich and soft. The final product has an alcohol concentration of averagely 17% (v/v) alcohol.

Mixed fruit wines

Different fruit juices can be mixed to prepare wine of desired quality. Combination of grape with other fruit is made so as to have vinosity of grape and flavour of the specific fruit used (Fowles, 1989). For different types of wine (dry white table and dry red table), fruit juices obtained from apple and white grape; apple, white grape, goose berry and pineapple; elder berries, black berries, pears, black currant and red grape respectively can be used in different proportions (for a detailed review on mixed fruit wines, see Joshi *et al*., 2000).

2.2.2 Authenticity of fermented beverages

Fermented beverages, including wine, must be authentic; that is without any form of adulteration or contamination. The general concept of the authenticity of a particular beverage can be defined as conformity to standard. Such a standard may arise from tradition, laws, reference compounds, industrial purchase specifications or other forms of written or non-written rules and/or traditions defining what a product is supposed to be in terms of origin, raw materials used, manufacturing process, aging, etc. (Martin *et al*., 1995).

A product is often non-authentic because inferior ingredients are used to cut production costs and increase profits (Martin *et al*., 1995).

Martin *et al*. (1995) hypothesised that food adulteration is probably as old as trade itself. They further added that through the ages, adulteration has evolved from relatively blatant forms, like the simple addition of water to milk, to highly sophisticated frauds where advanced chemistry is used, for example to isotopically label synthetic flavours purchased by beverage makers with carbon-13 and radioactive carbon-14 in order to imitate natural products.

Analytical methods such as isotopic analyses are in place to detect beverage adulterations. These methods are based on the principle that specific fruits have certain organic and non-organic compounds that can be detected in the juice and wine, either in their natural chemical forms or after transformation during alcoholic fermentation (Martin *et al*., 1995). These parameters and compounds include alcohol, phosphates, ash, sulphates, chlorides, sodium, potassium, calcium, magnesium, glycerol, glucose, fructose, organic acids (malic, citric, lactic, tartaric),

acetaldehyde, higher alcohols, etc., in their known concentrations (Sims and Bates, 1994).

2.3 BANANA CULTIVARS USED FOR JUICE AND WINE PRODUCTION

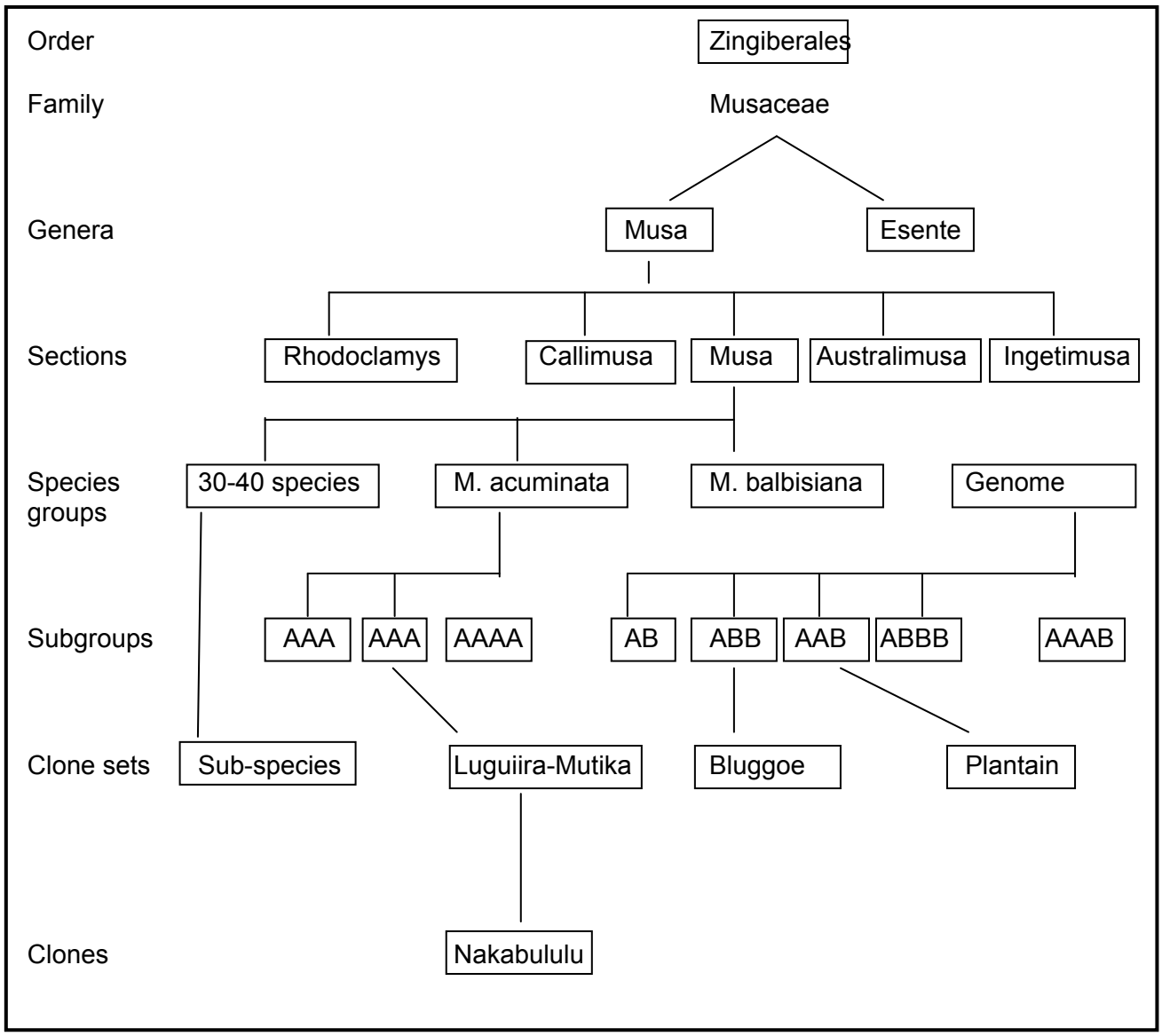

Figure 2.1: **The Classification of Bananas.** Source: Karamura, 1998

The East African highland 'beer' bananas consist mainly of the "Mbidde" group (Mbidde-AAA-EA genotype) i.e *Entanga, Enywamaizi, Imbululu, Kaitaluganda, Katalibwambuzi, Kibagampera, Mwanga, Namadhi, Namakumba, Nametsi* and *Nalukira.* The Kayinja (*Musa*, ABB genotype) cultivar is one of the Mbidde groups of bananas that are mostly used for juice extraction (Kyamuhangire *et al*., 2002) (see Figure 2.1 above). Other cultivars used for juice extraction include Kisubi (*Musa,* AB genotype) and, on rare occasions, the Dwarf Cavendish and Sweet *Ndiizi*.

2.3.1 Distribution and production of bananas

The ten major banana-producing countries produced about 75% of the total global banana production in 2004 (INFO COMM, 2005). India, Uganda, Brazil, Ecuador and China alone produced more than half of the total world banana crop. Regional distribution patterns and production levels have changed drastically over time. Whereas the Latin American and Caribbean regions dominated production up to the 1980s, the Asian region took the lead in banana production during the 1990s. The production of bananas in Africa remained relatively stable in these two decades. About 98% of the world's banana production is in developing countries and the usual destinations for banana exports are the developed countries. In 2004, a total of 130 countries produced bananas (INFO COMM, 2005). The banana production areas are listed in Appendix 2, and Tables 2.1, 2.2 and 2.3 provide information on the production and distribution of bananas.

Table 2.1: World production (1 000 t) of bananas and plantains (1992)

Region and country	Banana	Plantain	Total
Africa	6,937	19,937	26,874
East and Central	3,793	12,045	15,828
Burundi	1,445	-	1,445
Rwanda	-	2,900	2,900
Tanzania	794	794	1,588
Uganda	560	7,806	8,366
West and Central	3,154	7,892	11,046
Cameroon	100	860	960
Cote d'Ivoire	191	1,281	1,472
Nigeria	1,050	1,454	2,504
Zaire	406	2,300	2,706
America	21,939	6,422	28,361
Meso – America and Caribbean	8,347	1,510	9,857
Costa Rica	1,682	135	1,817
Honduras	1,086	182	1,268
Mexico	2,095	-	2,095
Panama	1,110	109	1,219
South America	13,592	4,912	18,504
Brazil	5,616	-	5,616
Colombia	1,950	2,573	4,523
Ecuador	3,995	975	4,970
Asia	20,230	762	20,992
China	2,651	-	2,651
India	7,500	-	7,500
Indonesia	2,500	-	2,500
Philippines	3,005	-	3,005
Oceania	1,287	6	1,295
World Total	**51,095**	**27,128**	**78,223**

Source: FAO, AGROSTAT, 1993

Table 2.2: World *Musa* production (1000 t) by use and genome group

Region	Cooking uses		Dessert uses	
	Plantains AAB	AAA Highland ABB	Cavendish Bananas	Other Bananas
Africa	7,784	11,498	945	2,791
East and Southern Africa	1,287	11,180	0	2,591
West and Central Africa	6,497	318	945	200
Latin America	6,302	83	12,494	4,050
Central America	1,576	41	6,239	0
South America	4,726	42	6,255	4,050
Asia	1	7,974	6,031	3,730
Oceania	0	25	5	0
Total	**15,086**	**19,580**	**19,475**	**10,571**

Source: INIBAP, 1993

Table 2.3: World supply (1000 t) and distribution of bananas and plantain (1992)

Region	Production	Imports	Exports	Supply	Waste	Processed	Food supply	Per capita consumption (kg)
Africa	26,874	23	273	26,624	3,253	4,701	17,098	25. 2
Latin America	28,361	379	8,741	19,999	3,125	79	14,883	32. 8
Meso America	9,857	86	4,256	5,846	943	27	4,261	28. 4
South America	18,504	293	4,485	14,312	2,178	52	10,622	34. 9
Asia	20,992	568	978	20,582	2,777	509	16,969	5. 5
Oceania	1,293	0	0	1,293	128	-	1,031	171. 5
Total	**78,223**	**970**	**9,992**	**69,201**	**9,279**	**5,289**	**54,633**	

Source: FAO, AGROSTAT, 1993

There are three main groups of bananas, *viz:* the cooking, juice and dessert types. The farmers in the banana-growing regions of the East African highlands, such as Rwanda, Burundi, Northern Tanzania, Uganda and Eastern Congo, commonly grow the banana juice-yielding cultivars (Kyamuhangire *et al*., 1996). Banana is the most extensively grown food crop in Uganda, covering more than 1.3 million hectares. The estimated yield of bananas in the country is about 8.5 million tons per annum. Bananas, especially the 'cooking cultivars', are a staple food for more than seven million people in Uganda (Aked and Kyamuhangire, 1996). Uganda's banana production represents 30% of the world production of cooking varieties and plantains and 11% of the total world banana production (FAO AGROSTAT, 2003). The majority of bananas grown in Uganda are East African highland varieties, which are found between 1000 and 2000 metres above sea level (Aked and Kyamuhangire, 1996).

Regarding international markets, INFO COMM (2005) reported that bananas are the main fruit in international trade and the most popular fruit in the world. In terms of export volumes, bananas are ranked first, while they rank second after citrus fruit in terms of value. According to statistical estimations by the UN's Food and Agriculture Organization (FAO AGROSTAT, 2003), total world exports of banana were 15.5 million tons. In many developing countries, bananas are considered a vital staple commodity and hence serve as a base for food security. Some of the main banana-producing countries, such as India, Uganda and Brazil, contribute little on the international market, and only about one fifth of total banana production is internationally traded (INFO COMM, 2005).

The banana industry is a very important source of income, employment and export earnings for the major banana-exporting countries, as well as the non-exporting ones. Banana exports are valued at US$4.7 billion per year (INFO COMM, 2005). Technologically, research and development (R&D) in the banana sector is needed to increase productivity and yields, as well as to improve the resistance of bananas to diseases and pests in order to reduce dependence on fungicides and pesticides (INFO COMM, 2005). Processing technologies are also required to add value to the banana products, especially to their beverages, which still has processing challenges in many developing countries.

2.3.2 Description of bananas

Bananas belong to the species *Musa acuminata* and *Musa balbisiana* (Figure 2.1). Bananas of the genus *Musa* are part of the family *Musaceae*, are considered to be derived from the wild species *acuminata* (AA) and *balbisiana* (BB). The plantain, or cooking banana, is classified as *Musa paradisiacal*. The Manila hemp is classified as *Musa textilis*.

Bananas are cultivated primarily for their fruit, and to a lesser extent for the production of fibre and as ornamental plants. As the bananas are mainly tall, upright, and fairly sturdy, they are often mistaken for trees, when the truth is the main or upright stem is called a *pseudo* stem, literally meaning "fake stem", which for some species can reach a height of up to 2–8 m, with leaves of up to 3.5 m in length. Each pseudo stem would produce a bunch of yellow, green, or even red bananas before dying and being replaced by another pseudostem. The banana fruit grows in hanging

clusters, with up to 20 fruits to a tier (called a hand) and 3-20 tiers to a bunch. The total of the hanging clusters is known as a bunch, or commercially as a "banana stem", and can weigh from 30–50 kg. The fruit averages 125 g, of which approximately 75% is water and 25% dry matter content. Each individual fruit (known as a banana or 'finger') has a protective outer layer (a peel or skin) with a fleshy edible inner portion (Wikipedia, 2008).

There are almost 1 000 varieties of bananas in the world, subdivided into 50 groups. The most commonly known banana is the Cavendish variety, which is produced mainly for export markets. Bananas are imported mainly by the European Union (33.9%), the United States of America (28.3%) and Japan (7.1%),which together accounted for 69.3% of world total imports in 2003 (INFO COMM, 2005). Other exports of banana are usually destined to the Russian Federation (4.2%), China (3.1%), Canada (2.9%) and the rest of the world (20.5%). The fruit of the plantain is larger, coarser and less sweet than the kinds that are generally eaten raw. The edible part of the banana contains, on average, 75% water, 10% carbohydrates, and about 1% fat, 3% protein and 10% fibre (see Appendix 5).

Traditional types and utilisation

The East African Highland bananas (AAA-EA genotype) are unique to the East African region. They are mainly grown by smallholders in complex mixed cropping systems and these bananas serve as the staple food for more than seven million people in Uganda, including two thirds of the urban population (Aked and Kyamuhangire, 1996). However, little research has been done on these types of bananas and almost no value-added products derived from them are available on the world markets.

The per capita calorie consumption of bananas compared to other carbohydrate sources in various countries is listed in Table 2.4. The growth rate in per capita consumption of bananas and plantains is given in Table 2.5. Various estimates of per capita consumption of bananas (*Matooke*) in Uganda are provided in Table 2.6 and the average monthly household consumption expenditure of bananas is given in Table 2.7. Africa ranked third with 18.9 kilograms per capita consumption of bananas and plantains after Oceania and Latin-America at 171.5 kg and 21.3kg per capita consumption respectively in 1992 as presented in Table 2.5. The per capita consumption in Kampala urban centre increased significantly between 1989 and

1993 as shown in Table 2.6. The average monthly household consumption expenditure of bananas is the highest in urban Kampala. This means that as a staple food, bananas compose a big portion of the household consumed items as indicated in Table 2.7.

Table 2.4: Daily per capita calorie consumption by principal carbohydrate source (1992)

Region/Country	Total	Vegetable	Cereals	Starchy roots	Bananas	Plantains
Africa	2,282	2,113	1,118	340	12	43
Rwanda	1,821	1,772	359	511	0	361
Tanzania	2,018	1,870	901	491	33	51
Uganda	2,159	2,011	403	581	42	408
Cameroon	1,981	1,848	810	342	0	152
Cote d'Ivoire	2,491	2,379	923	707	4	193
Zaire	2,060	2,002	320	1,157	15	109
Latin America	2,746	2,263	1,064	108	37	26
Dominican Rep.	2,286	1,966	707	77	81	140
Colombia	2,677	2,260	894	193	0	165
Brazil	2,824	2,187	949	138	37	30
Asia (Developing)	2,571	2,313	1,657	94	9	0
Oceania	2,675	2,333	655	585	296	0

Source: FAO, AGROSTAT, 1993

Table 2.5: Growth rate in per capita consumption of bananas and plantains, 1961-1992

Region	1992 Total consumption (1 000 t)	1992 per capita consumption (kg)	1961-1992 Growth rate per capita consumption (%)
Plantain			
Africa	12,089	18.9	0.0
Latin America	5,209	11.5	-0.3
Asia	673	0.2	0.0
Oceania	1	0.2	-5.7
Banana			
Producing:			
Africa	4,849	7.6	-0.7
Latin - America	9,674	21.3	-0.7
Asia	16,296	5.3	1.6
Oceania	1,030	171.5	-0.3
Importing:			
Western Europe	3,895	10.3	1.4
USA	2,968	11.7	1.7
Canada	371	13.5	1.4
Japan	646	5.2	3.0

Source: FAO, AGROSTAT, 1993

Table 2.6: Various estimates of per capita consumption (PCC) of bananas (*Matooke*) in Uganda

Source	Rural or Urban	Site	PCC (kg)
Household budget	Rural	Country	493.5
Survey (1989-90)	Urban	Country	305.5
	Urban	Kampala	229.0
Food balance sheets (1992)	Country	Country	178.4
Adupa (1993)	Rural	Western	517.5
	Rural	Mbale	386.5
	Urban	Masaka	445.3
	Urban	Kampala	354.0

Source: Adupa and Ngambeki, 1994

Table 2.7: Average monthly household consumption expenditure in urban Kampala

Food commodity	Household monthly expenditure (U Shs)	Household food expenditure (%)
Banana	4121	14.0
Maize	1619	5.5
Sweet potatoes	1218	4.1
Rice	1159	3.9
Cassava	917	3.1
Irish potatoes	322	1.1
Millet and sorghum	160	0.6

Source: National Household Budget Survey, (1989 – 1990)

2.3.3 Physical and technical characteristics of bananas

Bananas are perennial crops with high water succulent stems that give the high resistance to seasonal droughts. Both the 'cooking' and 'beer' banana varieties differ in physical characteristics. Both types mature in about three months from the flowering time on average. The "AAA-EA" *Mbidde* type of bananas has compacted bunches of fruit, with starchy banana fingers, but require an expert to differentiate them from the cooking varieties. The *Kisubi* banana trees are usually shorter than those of the cooking varieties, whereas the Sweet *Ndiizi* cultivar has smaller banana fingers and smaller stems in the mother crop. The average size of the banana fruit is 150 to 200 mm in length, with a diameter of 40 to 60 mm. The cooking variety (plantain) is usually harvested green, while the juice-yielding type can be harvested at full maturity, when the first 'fingers' near the stalk turn yellow. The ripe fruit has a unique flavour (taste and aroma). The 'cooking' cultivars are yellowish when peeled and rapidly undergo enzymatic oxidation. Bananas are very starchy fruit and are rich in carbohydrates. Harvesting is normally done by cutting the whole stalk that holds

the “banana bunch”. The bunch must be handled with care on landing to avoid injury to the fruit. Such injuries cause the quality of the bananas to deteriorate during post-harvest storage and cause big losses.

2.3.4 Chemical characteristics of bananas

The chemical composition of bananas varies and the variations are reported to be the result of many factors, including ecological location, nutrition, location on the bunch from which the banana fingers are sampled for analysis, and maturity of the fruit at harvest (IITA, 1993). This means that, even within the same cultivar, chemical analyses can differ. The different cultivars also differ considerably in terms of their chemical contents, such as starch, sugar, fat, minerals, acidity, water content, pectin and tannins. The starch content of ripe and unripe banana fruits was reported to be in the range of 0 - 28.6% (Sachiez Nieva *et al.*, 1970). Palmer, (1971) reported that unripe bananas have starch contents of 20 to 25%, although no mention was made of the particular cultivar that was tested. Kyamuhangire *et al.* (2002) analysed the composition of Kayinj banana juice (*Musa*, ABB genotype) extracted by enzymatic and mechanical methods (Table 2.8). The total soluble solids were higher in the enzyme-extracted juice than in the mechanically extracted juice, while the titratable acidity and viscosity were *vice versa*. Whereas the total sugar did not show much difference between the two methods of juice extraction used, however the sucrose, fructose and glucose components of the juices were found significantly different. The most dominant acid in banana juice was malic acid. It was established that the assimilable nitrogen concentration in enzyme-extracted Kayinja juice (0.8 gkg^{-1}) was significantly higher than in mechanically extracted juice (0.2 gkg^{-1}). The mineral composition of the banana juice analysed included potassium, magnesium, phosphorus, calcium and sodium. Potassium has been identified as the dominant mineral in banana juices (Kyamuhangire *et al.*, 2002, INFO COMM, 2005).

Unlike in many other fruits, where there is loss of acidity during ripening, it is reported (Palmer, 1971) that there is an increase in acid content during the ripening of bananas, with a decrease in pH.

Table 2.8. Analysis of the composition of banana juice extracted by enzymatic and mechanical methods

	Enzyme-extracted Juice	Mechanically extracted juice	LSD p=0.05
Pure juice yield (gkg^{-1})	604	541	3.8
Soluble solids(Brix)	34.9	30.7	1.3
pH	4.03	4.49	0.05
Titratable acidity(gkg^{-1})	5.4	3.3	0.26
Density (Mg m-3)	1.13	1.12	0.004
Viscosity($g\ m^{-1}s^{-1}$)	3.43	4.34	0.64
Nitrogen(gkg^{-1})	0.8	0.2	0.04
Sugars(gkg^{-1})			
Total sugar	259	253.3	1.78
Sucrose	1.3	165.2	1.01
Fructose	122.5	42.7	1.31
Glucose	130.7	44.8	0.75
Acids (gkg^{-1})			
Malic	6.1	5.9	0.44
Citric	3.7	3.8	0.38
Succinic	2.1	2.1	0.37
Minerals(gkg-1)			
Ash	10.1	12.2	2.8
Potassium	4.04	2.94	0.1
Magnesium	0.32	0.33	0.02
Phosphorus	0.23	0.24	0.03
Calcium	0.07	0.09	0.02
Sodium	0.03	0.02	0.01

Results are means of 20 experiments.

Source. Kyamuhangire *et al*. (2002).

The average moisture content in most banana varieties is 75% (Wikipedia, 2008). Ripe bananas contain approximately 0.5-1% pectin (Kawabata and Sawayama, 1974). In the banana pulp, insoluble protopectin decreases from about 0.5% to about 0.3% fruit weight and soluble pectin shows a corresponding increase during ripening (Palmer, 1971). Neubeck (1975) reported that fully ripe bananas might contain up to one-third to two-thirds of its pectin in soluble form. Also, Kotecha and Desai (1995) reported that ripe bananas contained 0.3% insoluble protopectin and 0.3% soluble pectin. Protopectin and pectin are the substances responsible for formation of hazes and turbidity respectively in the beverages extracted from ripe bananas. Tannins as chemical components of bananas are of great significance for flavour. When tannins react with proteins and glycoproteins on the surface of the tongue and buccal mucosa, they cause a drying and puckering sensation known as “astringency”. For the suitability for consumption, the end use of a particular cultivar is governed, in part, by the astringency of the green banana fruit. The medium and highly astringent (“beer”) cultivars are only eaten during famines (Aked, 1995). A bit of astringent taste

would be desirable for banana winemaking. Total acidity in low levels and sugar for sweetness are also desirable and contribute to the flavour of banana beverages.

2.3.5 Volatile components from fruits

The aroma and taste (flavour) of fruits improve with ripeness. The volatile compounds in fruits can be easily analysed in fruit wine distillates with help of gas chromatography. Loss of volatiles in the fruit beverage can be increased by heating or boiling juices / wines. The longer one heat fruit beverages, the more volatile components will be lost depending on temperature used, more especially if the heating is done in an open system (Kyamuhangire and Pehrson, 1999). The volatile compounds found in different wines are presented in Appendix 6. Those volatiles include methanol, higher alcohols, esters, acetaldehydes and organic acids.

2.3.6 Nutrition facts about bananas

Bananas have various nutritional substances. It is reported (http://answers.yahoo.com) that a serving size of 3.5 oz (99.23g) of banana supplies the following:

Total Fat 0.7g ,1%
Saturated Fat 0.1g ,0%
Monounsaturated Fat 0.4g
Polyunsaturated Fat 0.2g
Sodium 1.4mg, 0%
Potassium 486.9mg, 14%
Total Carbohydrate 31.1g, 10%
Dietary fibre 3.5g, 14%
Sugars 16.6g
Protein 1.5g, 3%
Vitamin A 2%
Vitamin C 20%
Calcium 1%
Iron 2%
Est. percent of calories from:
Fat 7.7%
Carbohydrates, 151.7%
Protein 7.3%.

Nutritionally, bananas are considered to be good for the treatment of gastric ulcers and diarrhoea and, because they contain vitamin A, bananas act as a digestive aid. Due to their high content of vitamin B6, bananas help in the reduction of stress and

anxiety and the high content of carbohydrates makes bananas a very good source of energy for people practicing sports (INFO COMM, 2005).

2.4 WINE FERMENTATION

Wine is desired globally as an alcoholic beverage in different forms. Wine can be processed as a table or dessert, dry or sweet, still or sparkling, natural or fortified form. United States regulations define wine as containing between 7 and 24% (v/v) alcohol. Beers and ales have lower alcohol levels than wine, whereas liquor and spirits are much stronger alcoholic beverages. Fermentation of wine occurs spontaneously by native yeasts and by inoculation with selected yeasts. Fermentations are metabolic processes which bring about chemical changes in organic substrates through the action of enzymes produced by microorganisms (for more details about wine fermentations, see Amerine *et al*., 1980; Zoecklein *et al*., 1995; Boulton *et al*., 1996; Margalit, 1997; Jackson, 2000 and Ribéreau-Gayon *et al*., 2000).

However, compounds / substances may be added during wine fermentation for various reasons. These compounds/substances include the addition of:

i) diammonium phosphate (DAP) to limit production of hydrogen sulphide.
ii) tannin to enhance mouthfeel characteristics.
iii) expanded cellulose to provide sites for absorbing yeast secreted toxins (for a detailed review on yeast fermentation, stuck fermentations and malolactic fermentation, see Iland *et al*., 2000).

2.4.1 Alcoholic fermentation

Fermentation is an energy-releasing form of metabolism in which both the substrate and by-product are organic compounds. The free energy (transported by ATP) is used in the synthesis of products and by-products with release of some heat. Fermentation differs from respiration by not requiring the involvement of molecular oxygen. Under aerobic conditions (respiration), glucose is degraded to give carbon dioxide, water and energy whereas alcoholic fermentation degrades glucose to give

ethanol, carbon dioxide and energy. Under anaerobic conditions, yeast cells metabolise glucose by means of the Embden-Meyerhof-Parnas route, as follows:

$$C_6H_{12}O_6 \longrightarrow 2CH_3.\ CH_2.\ OH+2CO_2+56\ kcal$$

The above reaction takes place with the aid of several enzymes and intermediate products during glycolysis and alcoholic fermentation. In alcoholic fermentation, it is not pyruvate but rather acetaldehyde, its decarboxylation product, that serves as the terminal electron acceptor (for a detailed review on biochemistry of alcoholic fermentation, see Ribereau-Gayon *et al*., 2000).

In addition to enzymes that occur in pre-and post-fermentation practices, there are at least eleven different enzymes involved in the fermentation kinetics and the twelfth enzyme, alcohol dehydrogenase catalyses the final ethanol yield.
Although many fermentative microorganisms exist, *Saccharomyces cerevisiae* is adapted best for alcoholic fermentation (Jackson, 2000).

2.4.2 Yeasts

S. cerevisiae strains are recognized as not toxic or pathogenic and are normally used in human diets. The protein content of yeasts rarely exceeds 60%, but yeasts have a reasonable concentration of essential amino acids such as lysine, tryptophan and threonine. Yeasts are also rich in vitamins (B group), and their nucleic acid content ranges from 4 to 10%. Yeasts, being larger than bacteria, the size facilitate their easy isolation and separation. However, the specific growth rate of yeasts with a generation time of two to five hours is relatively slow, compared to that of bacteria (Boze *et al*., 1992).

Most yeast strains used in the food industry belong to the species *Saccharomyces cerevisiae*, *S. bayanus* and *S. uvarum*. Strains of the yeast *S. cerevisiae* are the most commonly used in alcoholic beverage fermentations. The majority of winemakers use commercial strains of *S. cerevisiae*, although the use of *Schizosaccharomyces pombe* has been reported in the production of rum.

The wine-related yeasts are those yeasts that are found on grapes or in vineyards, in table or dessert wines or are associated with wineries or winery equipment. Comprehensive detail of yeasts strains have been published (Kunkee and Amerine, 1968; Kunkee and Amerine, 1970; Kunkee and Bisson, 1993).

Wild yeast

Originally, wine was not deliberately inoculated but rather would be fermented spontaneously. “Wild yeasts” are those native yeasts found in fruits or on process equipment, which, if not hindered, may take part in wine fermentations – at least at the onset. Wild yeasts include *Kloeckera, Hanseniaspora* and *Debaryomyces.* However, the most frequently isolated wild species are the apiculate *Hanseniaspora uvarum* and its asexual counterpart, *Kloeckera apiculata*, which account for over 50% of the total wild species. Other common species include *Metschnikowia pulcherrima* and its asexual counterpart, *Candida pulcherrima*. Less frequent isolates are *Pichia membranefaciens, Hansula anomala, Candida stellata, Cryptococcus* spp. and *Rhodotorula* spp. Endogenous microflora are responsible for spontaneous fermentations (for a detailed review on wild yeasts, see Du Toit and Pretorius, 2000).

Saccharomyces cerevisiae yeast

This yeast species is universally known as brewer’s or wine yeast. Generally, wine yeasts are many strains of *Saccharomyces*, which not only can carry out complete fermentation of the fruit juice or any other high sugar-containing medium, but also provide the fermented product with pleasant flavours. Morphologically, *Saccharomyces* spp. appears spherical to ellipsoidal in shape, with approximate dimensions of 8 x 7µm, depending on the strain and growth medium. Most *S. cerevisiae* strains are capable of producing alcohol levels of up to 16%, while the use of supplemented, “syruped” fermentations may facilitate the yeast to produce 18% or more alcohol in grape wine. *S. cerevisiae* is one of the three popular species used in the food industries. The other two common yeasts are *S. bayanus* and *S. uvarum* (for a detailed review on yeast and its importance, see Lambrechts and Pretorius, 2000).

2.4.3 Factors that affect fermentation

In this section, the factors which affect the process of alcoholic fermentation in fruit musts are discussed. The factors that significantly affect fermentation include:

Juice clarification

White juice is typically clarified by settling before fermentation to remove high levels of suspended solids and maintain desired flavour and fruitiness. At high levels, suspended solids in the must can increase hydrogen sulphide production (Singleton *et al*., 1975). Boulton *et al*. (1996) confirmed that there are several reasons for the clarification of white juices prior to fermentation or storage. These reasons include:

(1) a major proportion of the oxidative enzyme activity, which is associated with the pulp and grape skin fragments;
(2) the incidence of mould and wild flora, which is highest on the skin fragments;
(3) the laccase from botrytis or other moulds, which will be present in both pulp and skin tissue;
(4) elemental sulphur and other vineyard residues, which will be more associated with skin and pulp;
(5) some evidence of esterase activity within the grape tissue which reduces the accumulation of esters produced by the yeast during fermentation.

For all the above reasons, it necessitates some degree of clarification (opalescence) of white grape juices before fermentation. Several methods can be used to clarify juices. The reduction of suspended grape solids to levels of 1 to 2% by volume prior to fermentation is a common practice and is supported by sensory evaluations (Singleton *et al*., 1975).

However, for most purposes an opalescent juice is adequate, water-clear juice is not necessary and is more likely to lead to fermentation difficulties when juices have a low nutrient status (Boulton *et al*., 1996). This means that yeast growth will not be optimum as required in the biomass due to many nutrients removed during juice clarification. Such fermentation may end up in a lag fermentation or stuck fermentation when there is a high nutrient status (suspended solids) in the juice or must.

Clarifying juice removes most of the suspended material including colloidal sulphur, thus avoiding major hydrogen sulphide problems (Amerine *et al*., 1980). Particles in suspension, either in forming a haze or dispersed through the liquid, not only spoil the presentation of wine but also affect the flavour (Ribéreau-Gayon *et al*., 2000). Production of fusel alcohols is usually associated with juice containing high levels of suspended solids.

The elimination of particulate matter also eliminates most of the micro organisms adhering on them. This successful removal of wild yeasts, acetic acid bacteria and lactic acid bacteria, leads to a more controlled or predictable fermentation when inoculated with pure selected yeast starter culture, and eventually may yield wine of a higher quality. On the other hand, it is also well established that wines made from clarified juices are easier to clarify (Amerine *et al*., 1980).

Free available nitrogen concentration

The major amino acids in the average grape juice are: proline, arginine, alanine, glutamate, glutamine, serine and threonine (Boulton *et al*., 1996). Ammonium ion levels may also be high in the grape depending upon the variety and time of harvest. The presence of nitrogenous materials in must supplements the amount of nitrogen assimilated by yeasts. During yeast growth and multiplication (exponential phase), the nitrogen content of the must decreases. Most of the nitrogen requirements of yeast are met through the uptake of amino acids. These nitrogen-containing compounds of fruits and wine are of great importance for the growth of yeast and bacteria, and hence for the fermentation rate and aroma compounds production. European data summarised by Amerine (1954) sets the average amount of total nitrogen required in grape must for wine making at 0.10 to 0.77 g/L. Other studies showed that although the total assimilable nitrogen content in grapes (all amino acids, except proline which is not utilized by yeast, plus ammonia) is about 500-1000 mg/L, supplementation of nitrogen (with diammonium phosphate and/or amino acids) increases fermentation rate when addition was as high as 800-2000 mg/L (Vos *et al*., 1979; Vos *et al*., 1980; Monterino and Bisson, 1992).

For a number of fruits, the nitrogen content is insufficient and supplements are made by the addition of ammonium phosphate or of diammonium phosphate (DAP) with

amounts to be added determined before fermentation. Glutamate is also another preferred nitrogen source because it is utilized directly for biosynthesis (Boulton *et al*., 1996). Glutamine can generate both ammonium ion and glutamate. Nitrogen–containing compounds in grape juice may meet one of three fates: (1) Utilized directly in biosynthesis; (2) converted to a related compound and utilized in biosynthesis; or (3) degraded releasing nitrogen either as free ammonium ion or as bound nitrogen via a transamination reaction (Boulton *et al*., 1996).

Temperature

Temperature affects fermentation, especially affecting yeast growth and activity. The temperature range in which fermentation is favoured has been given in different ranges by different researchers. According to Schanderl (1959), the optimum fermentation temperature by most wine yeasts is between 22^0C (71.6^0F) and 27^0C (80.6^0F). Margalit (1997) reported that fermentation can take place in the temperature range from 5°C to 38°C (41°F to 100°F) because below and above that temperature range, yeast enzymes may not function. Margalit further advises not to allow the fermentation temperature to exceed 33°C, as fermentation may stop above this temperature. The usual period for a cool fermentation to dryness (at about 8 to 10°C) may take three to four weeks, whereas warm fermentation (25°C to 30°C) may come to completion in four to six days. White wines are fermented at the lower range, usually at 8^0C-14^0C while the red wines are fermented at the higher end, usually at 25^0C-30^0C (Margalit, 1997). These temperature ranges seem not to apply to all fermentations depending on different wine styles and conditions. The optimum temperature for yeast growth has been reported to be close to 30°C (Reed and Nagodawithana, 1995). For this reason, fermentation of the must is faster at higher temperatures due more active yeast cells and greater activity of the fermentation pathway enzymes. The rise in temperature of the must also affects the composition of wine by causing some loss in alcohol. CO_2 formed during fermentation carries with it not only heat, but also ethanol and other volatile substances. Losses in ethanol are reported to be in the range of 0.5 to 1.5% of the alcohol formed (Margalit, 1997). It should be noted that the temperature range for various fermentation is quite wide but the choice of temperature to be used for a particular product depends on the rate (slow or fast) of fermentation preferred and quality of fermented product (with little or much flavour) desired.

Juice composition

Pressed juice differs in several aspects from free-run juice. The factors of pressed juices that import on the desirability include suspended solids, higher phenol and tannin concentrations, lower acidity, higher pH and a higher concentration of polysaccharides. Pressed juices prove to have higher levels of oxidative enzymes due to the solids content being higher, and they brown more readily because of the presence of phenol substrates. There is concern about the polysaccharides (pectin, glucan, xylan, etc), which affect particle settling and cause filtration problems in case of processing clarified fruit beverages.

Generally, fruit juices contain all the nutrients required for unstressed and healthy fermentation. One limiting factor may be nitrogen deficiency, and juice e supplemented by diammonium phosphate (DAP) and/or amino acids. The sugar concentration of the juice has a big influence on yeast growth during the lag-phase period of fermentation. Most fruit juices have sufficient sugar required for fermentation to take place but in case the sugar is not enough, sucrose may be added. It is reported (Margalit, 1997) that the optimum yeast growth during the lag period appears between 15 and 20°Brix.

Musts with lower concentrations of sugar start to ferment fast and the sugar is fermented to completion, whereas musts with high sugar content ferment slowly and may be incomplete. High sugar concentrations inhibit fermentation by high osmotic pressure, which draws water from the yeast cells in particular (Margalit, 1997).

Sulphur dioxide

While sulphur dioxide (SO_2) is universally used for its antiseptic and antioxidative properties, there is general agreement that the odour of free sulphur dioxide is undesirable and therefore, there has been a long search for a substitute. Owing to the multiple effects of sulphur dioxide, a single substitute remains unlikely (Amerine *et al*., 1980). Ascorbic acid has been widely used in Germany as an antioxidant. However, it was found that small amounts of free sulphur dioxide could not be replaced completely by ascorbic acid because ascorbic acid does not have antiseptic properties. Sulphur dioxide tolerance differs in various yeast species and strains.

The normal *S. cerevisiae* strains are reported to tolerate up to 4 mg/L of undissociated SO_2. Free SO_2 added to must is usually bound during fermentation. This bound SO_2 has hardly any microbicidal effect. An invigilator effect thus is expected if free SO_2 is available in the must.

Aeration

Although fermentation is anaerobic, the use of oxygen in the first phase is of crucial necessity. It is needed for synthesising the steriod *ergosterol* and unsaturated fatty acid- *oleic acid* ($C_{18}H_{34}O_2$) for cell membrane building (Margalit,1997). In the absence of oxygen, the yeast cannot multiply and increase its biomass to that required to complete the fermentation. The residual oxygen content at the time of yeast inoculation has a major influence on the viability of cells at the end of cell division and on their death rate in the remainder of fermentation (Ribereau-Gayon *et al*., 1982).

The solubility of oxygen from the air into juice and wine is about 8 to 9 mg/L at 20°C due to the partial pressure of oxygen being approximately 20% of the air. In comparison, the oxygen solubility rises to about 40 mg/L with use of oxygen headspace (Boulton *et al*., 1996).

It is assumed that the phenols involved in the oxidation and browning of wine are substrates for enzyme activity. One approach to handling the control of oxidation at the juice level has been to deliberately aerate the juice of white grapes prior to fermentation in a process sometimes referred to a *hyperoxidation.* The aim of this approach is to oxidise many of the phenolic components which would normally be the substrates for chemical oxidation (and browning) in the subsequent wine. The brown pigments formed by this action will generally be absorbed to solids and be removed by precipitation during fermentation, leaving only the golden, straw-coloured pigments in the wine (Cheynier *et al*., 1990).

Acidity and pH

Juices and musts that do not possess the required acidity and pH may be adjusted to the desired levels before fermentation. Acidity adjustment is achieved either by acid

additions or acidity reduction. It is generally accepted that a juice (or must) treatment is preferred to a wine treatment and this is especially so for the carbonate deacidifications or unless concurrent alcoholic and malolactic fermentations are desired (Boulton *et al*., 1996).The adjustments will generally be based on target values for titratable acidity and pH rather than by sensory evaluation, due to the influence of sugar levels in the juice. The ability to obtain the desired level of acidity adjustment is further reported to be influenced by the natural variation in the buffer capacity of various juices. The buffer capacity is primarily proportional to the concentrations of tartaric and malic acids and will decrease with reductions in their levels. Acidification also plays a role in limiting the growth of spoilage microorganisms. The acids are very important in maintaining the pH low enough so as to inhibit the growth of many undesirable bacteria, thus giving a growth advantage to wine yeasts (Amerine *et al*., 1980). However, if the pH is very low, 3.0 or lower, fermentation rate is somewhat reduced.

The lag-phase period for yeast growth is reported to be very sensitive to both pH and temperature. This pH is a vital factor in the acclimatisation of yeasts to the wine media and the period until the start of yeast multiplication is thus longer, the lower the pH (Margalit, 1997).

In some situations, juice displays both high pH and high acidity, a situation common in grapes from cool climatic regions, where the fruit have high malic acid and potassium contents. Deacidifications can be done instead of acidification after the fermentation process on the basis of the actual need. Amelioration is also a means of de-acidifying, involving the dilution of acidity using water. However, amelioration is illegal in several countries. Such amelioration also reduces the sugar content and necessitates the addition of sugar to readjust the ^{o}Brix to the required level.

Generally, it is difficult to recommend specific optimum acidity and pH values for different fruit juices. This is because the ideal characteristics of wine are very much dependent on the style and preferences of the particular wine consumer. Nevertheless, typical ranges for titratable (total) acidity in grape juices are 5.5 to 9 g/L as tartaric acid and pH 3.1 to 3.4 (Boulton *et al*., 1996).

Ethanol and carbon dioxide

Alcohol itself has an inhibiting effect on fermentation which increases with temperature (Amerine *et al*., 1980). At a certain concentration, ethanol, begins to inhibit (depending on yeast strain alcohol tolerance) the fermentation rate and finally halts it completely. A slight inhibition of yeast growth occurs when the alcohol concentration of the fermenting must reaches 2% (v/v). In dry wine (without the combination of alcohol-sugar), full inhibition is reached at a concentration of 16-17% (v/v) (Margalit, 1997). The effect of high concentrations of alcohol on the fermentation is to reduce the intracellular diffusion of the internally produced ethanol (by fermentation) towards the outside of the cells and to the external medium. As the external concentration (in the medium) becomes higher, the diffusion rate gets lower, and at a certain point, the accumulated internal alcohol, will cause interference with membrane structure and substrate transport. The tolerance of yeast strains to alcohol concentration is quite wide, and may depend on other medium conditions such as sugar, temperature, sulphur dioxide and other by-products in the medium. Furthermore, the alcohol tolerance of particular yeast depends not only on its tolerance to the internal ethanol pool, but also on the plasma membranes, which in turn depends on the properties of the substrate.

However, the effect of carbon dioxide on alcoholic fermentation is often neglected (Amerine *et al*., 1980). Considering carbon dioxide and pressure, Amerine and others reported that carbon dioxide content of 15 g/L (about 7.2 atm) essentially stopped yeast growth. The carbon dioxide effect on yeast growth did not prevent alcoholic fermentation. A much higher carbon dioxide pressure, up to 30 atm, was necessary to halt alcoholic fermentation. The inhibiting effect of CO_2 is used to control fermentation rates in a pressure-controlled fermentation (Troost, 1988).

Minerals

The normal process of alcoholic fermentation requires minerals such as magnesium, potassium, zinc, cobalt, iodine, iron, calcium and anions of phosphorus and sulphur. For growth alone, yeasts require copper, iron, magnesium, phosphorus and sulphur. Adequate amounts are supplied by grape juice and, apart from phosphate; most other fruit juices are also able to supply these minerals (Amerine *et al*., 1980).

Phosphorus in the form of phosphate anion ($H_2PO_4^-$) is essential in all energy transfer processes of yeast growth. Its natural concentration in grape juice is adequate for normal yeast fermentation. By addition of DAP as nitrogen source, phosphate is added too. Cations (such as K^+, Mg^{2+}, Zn^{2+}, Mn^{2+}) are important factors in enzymatic activity during fermentation, and deficiency in any of them may have a serious impact on its rate. But due to their natural content in grape juice, it is practically not of any concern (Margalit, 1997). Potassium and magnesium are adequately present in banana juice (Kyamuhangire *et al*., 2002).

Other fermentation inhibiting factors

Other factors which are important inhibitors of fermentation include the presence of fatty acids (mainly octanoic and decanoic) and killer yeasts (for detailed review on these fermentation inhibitors, see Margalit, 1997).

2.5 FERMENTATION BY-PRODUCTS

Ethanol is the main product of alcoholic fermentation, and other by-products of fermentation occur in small but significant amounts. The other alcohols, organic acids and other by-products of importance in fermentation are considered briefly below.

2.5.1 Esters

Generally, esters have fruity and floral impact characteristics that play a vital role in the sensory properties of wines. Esters have three sources:

(1) Produced by the grapes in very small amounts.
(2) Produced during fermentation process (neutral esters).
(3) During aging of wine as a very slow chemical esterification (acid esters).

All these esters constitute a group of aroma compounds in alcoholic beverages.
The neutral esters are considered as volatile aroma components, and are called volatile esters. The acid esters, mainly tartaric and malic, are called also non-volatile esters (Margalit, 1997). The concentrations of the volatile esters in wine are low and

their organoleptic perception is described as a fruity-type of aroma, except for ethyl acetate which has the highest concentration in wines. The ethyl acetate contribution to the wine quality is considered negative, i.e. it is always accompanied by acetic acid and contributes the vinegar flavour to wine at concentration of 120-160 mg/L compared to acetic acid noticeable by taste as a hard sourness at about 700 mg/L (Margalit, 1997).

Esters are produced by yeast during fermentation in a reaction between alcohols and acyl CoA molecules. The amount of esters that are formed will depend on the relative abundance of the corresponding alcohol and acyl CoA produced by the yeast (Lea and Piggott, 1995).

Under certain conditions, such as anaerobiosis, unsaturated fatty acids and sterols cannot be produced and this inhibits normal membrane formation. In such conditions, organic acids become available and are converted into esters, which are secreted into the surrounding medium. Therefore, conditions of restricted growth, such as a lack of aeration, lead to increased ester formation. In conditions that favour a high ethanol yield, the growth of yeast in a well-aerated environment can totally reduce ester formation (Berry and Watson, 1987).

2.5.2 Aldehydes

Aldehydes and ketones are formed during alcoholic fermentation. The main representative of aldehydes in wine is acetaldehyde. Acetaldehyde is a major component in the fermentation chain process which normally is reduced to become the final product-ethanol (Margalit, 1997).

Acetaldehyde is a by-product of alcoholic fermentation that is formed from ethanol in the presence of the enzyme alcohol dehydrogenase. As wines age, acetaldehyde levels increase due to chemical oxidation of ethanol, and in case of improperly stored wines, growth of oxidative yeasts and bacteria at the wine's surface (Zoecklein *et al*., 1995). The film referred to as "mycodema" in some literature exists as a mixed population of several species including *Pichia, Candida, Hansenula* and oxidatively growing *Saccharomyces.* However, oxidative metabolism may be exploited in sherry production where acetaldehyde levels may exceed 500mg/L (Zoecklein *et al*., 1995).

Ough and Amerine, 1958) reported acetaldehyde accumulations of up 1 000 mg/L when floral yeast (*Saccharomyces fermentati*) is grown under aerobic conditions with slight pressure and occasional agitation. It has been reported further that the "faded" odour of newly bottled, low SO_2 wines is due to the temporary accumulation of acetaldehyde in wine (Amerine *et al*., 1982). Acetaldehyde is well established as an intermediate in bacterial formation of acetic acid in wine (Bartowsky and Henschke, 2004; Margalit, 1997; Zoecklein *et al*., 1995; Boulton *et al*., 1996; Ribereau-Gayon *et al*., 2000; Jackson, 2000 and Amerine *et al*., 1980).

2.5.3 Higher alcohols

The term higher alcohols refers to those alcohols possessing more than two carbon atoms and with a higher molecular weight and boiling point than ethanol. Higher alcohols are quantitatively the largest group of aroma compounds in alcoholic beverages and are secondary products of alcoholic fermentation (Amerine *et al*., 1980). The major source of higher alcohols is amino acids which by a sequence process of trans-amination, decarboxylation and reduction are transformed into alcohols (Margalit, 1997). However, the higher alcohols are also formed from sugars, utilizing part of the enzymatic pathways needed for formation of the corresponding amino acids (Boulton *et al*., 1996).

These alcohols of more than two carbons are also termed fusel oils and can be recognised by their strong pungent smell and taste (Lambrechts and Pretorius, 2000). When the concentrations of higher alcohols exceed 400 mg/L, they are regarded as a negative influence on the quality of wine (Rapp and Mandery, 1986). However, their overall presence in grape wine covers a wide range: from a concentration slightly less than 100 mg/L to a concentration higher than 500 mg/L (Nykänen, 1986). Higher alcohols can be of major importance in wine distillates, in which they are much more concentrated (Boulton *et al*., 1996). The most commonly encountered higher alcohols, in order of the amounts produced, include isoamyl alcohol (3-methyl butanol), "active amyl" alcohol (2-methyl butanol), isobutyl (2-methyl propanol) and n-propyl alcohol (Zoecklein *et al*., 1995; Boulton *et al*., 1996). The higher alcohols themselves have little impact on the sensory properties of wine, but they can be of major importance in wine distillates, in which they are much more concentrated (Boulton *et al*., 1996).

The main factors contributing to the formation of higher alcohol (fusel oils) include:

(a) Yeasts – Native yeast species produce higher alcohols, in contrast to pure-culture fermentations, and *Hansenula anomala*, for example, has been reported to produce fusel oils even in the absence of complete fermentation (Rankine, 1967).

(b) Temperature – Rankine (1967) reported that a rise in fermentation temperature from 15^{o}C to 25^{o}C encourages the formation of isobutyl by about 39%, whereas the active amyl level increased by 24%. The formation of n-propanol, however, was reported to decrease by 17%.

(c) Oxygen levels – The formation of higher alcohols in general and of isobutyl alcohol in particular increases in aerated musts. Similar production increases in suspended solids and particle size have been studied and seem to be most likely as a result of the absorption and retention of oxygen within the solids matrix (Khingshirn, *et al*., 1987).

(d) pH – Rankine (1967) reported that, in pure-culture fermentations utilising four yeast strains, an increase in pH from 3.0 to 4.2 resulted in increased production of active amyl and iso-amyl (28%), isobutanol (85%) and n-propanol (11%).

(e) Nutritional status of must – According to studies by Vos and others (1978) increases in assimilable nitrogen result in lower levels of higher alcohols in wine, with the exception of n-propanol (a result of pyruvate-acetyl CoA condensation). In this regard, the winemaker has some possibilities for control over the formation of higher alcohols, most importantly by taking into account the content of the nitrogenous components (Boulton *et al*., 1996).

2.5.4 Glycerol

Glycerol is normally a by-product of alcoholic fermentation resulting from the reduction of dihydroxyacetone phosphate. It is reported present in wine even though the glycerol content of the grape juice is low. United States wines are reported to have levels of glycerol ranging from 1.9 to 14.7 g/L, with a confirmed average of 7.2 g/L (Amerine *et al*., 1982). The main factors that affect the formation of glycerol include fermentation temperatures, yeasts, fruit condition and sulphur dioxide.

2.6 MICROBIAL SPOILAGE IN WINE

The winemaking process is a complex ecological niche where the biochemistry and interaction of yeasts, bacteria, fungi and the viruses play a pivotal role in the final product. These microorganisms involved are at the core of the winemaking process, whether for good or ill; they affect the quality of wine and determine the economic balance sheet of wine production (Du Toit and Pretorius, 2000). The main microorganisms associated with wine spoilage are yeasts, acetic acid bacteria and lactic acid bacteria. Since yeasts can generally resist extreme conditions better then bacteria, they are often found in low pH products and products containing high levels of preservatives (Deak *et al*., 2000).

Winemaking processes include multiple stages at which microbial spoilage is likely to occur. The first stage involves the fruit material to be processed and equipment to be used. One must attempt to reduce the numbers of Microbes in the juice and on the equipment. This is achieved through processing the pulp by applying food hygiene practices and following the hazard analysis critical control point (HACCP) system. The second stage of microbial spoilage may occur during fermentation because at this stage, the fruit juice contains both the natural flora of the fruit and flora that may be harboured by the wine cellar and its equipment. The microbial spoilage ends up altering the quality and hygienic status of wine. This may render the wine unacceptable, since the spoilage can include bitterness and off-flavours (mousiness, ester taint, phenolic, vinegary, buttery, etc.), as well as cosmetic problems such as turbidity, viscosity, sediment and film formation. The major spoilage organisms of the yeast genera include *Brettanomyces, Candida, Hanseniaspora, Pichia* and *Zygosaccharomyces*. The genera of lactic acid bacteria include *Lactobacillus, Leuconostoc* and *Pediococcus,* while the acetic acid bacteria genera are *Acetobacter* and *Gluconobacter* (Du Toit and Pretorius, 2002).

The spoilage caused in wine by yeasts is important because they cause re-fermentation, ester formation, hydrogen sulphide and volatile sulphur compounds, volatile acidity, the formation of volatile phenols, mousiness, film formation, deacidification and the formation of ethyl carbamate. *Saccharomyces* is regarded as spoilage organism only if it is found in the wrong place at the wrong time (e.g. in a

bottle of semi-sweet wine) causing re-fermentation. *Schizosaccharomyces pombe* has been associated with wine spoilage when growing in bottled wine and forming a sediment at the bottom of the bottle (Boulton *et al*., 1996). The yeast *Zaygosaccharomyces bailii* is one of the major wine spoilage yeasts, re-fermenting juice or wine during storage (Sponholz, 1993; Fugelsang, 1996, 1998). Yeasts *Hansenula anomala, Kloekera apiculata* and *Hanseniaspora uvarum* are associated with ester taint of faulty wines, which correlates with large amounts of acetic acid. These three species are associated with grape juice and result in spoilage at the early stages of alcoholic fermentation (Fleet 1990; Boulton *et al*., 1996). The ester taint can be linked to the presence of ethyl acetate and methyl butyl acetate, which are most prominent in wines possessing this off-flavour (Sponhols *et al*., 1990; Boulton *et al*., 1996). Wines with concentrations of >200 mg/L ethyl acetate and 0.6 mg/L of acetate are regarded as spoiled (Du Toit & Pretorius, 2000). Hydrogen sulphide (H_2S) is produced by yeasts during fermentation through the sulphate reduction pathway and has a flavour threshold of 50-80 mg/L and when exceeding this value will produce the rotten-egg off-flavour (Wenzel *et al*., 1980). The ability of yeasts to produce H_2S varies between strains and is influenced by environmental factors such as must composition (solids, vitamins and free amino nitrogen), fermentation temperature, wine pH and the use of fungicides containing elemental sulphur (Henschke and Jiranek, 1993; Rauhut, 1993; Zoecklein *et al*., 1995; Rauhut *et al*., 1996). It is thus important to select *S. cerevisiae* strains that produce limited amounts of hydrogen sulphide to reduce the risks of wine containing high levels volatile sulphur compounds that will render the wine quality unacceptable (review Du Toit and Pretorius, 2000).

The spoilage caused by lactic acid bacteria (LAB) is associated particularly with acetification of the wine through the production of acetic acid, mousy taints; stuck fermentations due to high levels of acetic acid produced by *Lactobacillus kunkeei*, flocculent growth, bitterness, ropiness, buttery flavour and increased viscosity of the wine. Yeasts involved in acetification of wine above objectionable levels include *Brettanomyces* and its anamorph *Dekkera*, *P. anomala, K. apiculata* and *Candida krusei* (Shimazu and Watanobe, 1981; Zoecklein *et al*., 1995).

The main spoilage caused by acetic acid bacteria (AAB) is associated with oxidation of the ethanol to acetaldehyde and eventually acetic acid, the production of ethyl acetate and acetoin, as well as the metabolism of glycerol to dihydroxyacetone.

Gram-negative acetic acid bacteria require oxygen for growth. They carry out incomplete oxidation of alcohols, leading to the accumulation of organic acids as end products (Bartowsky and Henschke, 2004; Amerine *et al*., 1980).

Even though the optimum pH for the growth of AAB is 5.5 to 6.3 (Holt *et al*., 1994), they are able to survive at wine pH (3.0-4.0). A pH of 3.3 or lower is inhibitory to most lactic acid bacteria, but not to AAB (Vaughn, 1955). Acetic acid bacteria have been isolated from Australian wines with a pH of 3.02 to 3.85 (Drysdale and Fleet, 1985). Although AAB are able to grow at lower than wine pH, the growth of AAB was shown to be much greater in South African red wines with a higher pH (<3.75 compared to 3.5) (Du Toit and Lambrechts, 2002; Du Toit and Pretorius, 2002). The presence of sulphur dioxide (SO_2) in wine should prevent the growth of AAB; however, the form of SO_2 is important for bactericidal action, the molecular form being the most effective (Bartowsky and Henschke, 2004). Studies have shown that AAB are capable of growing in wine containing 20 mg/L of free SO_2 (Joyeux *et al*., 1984), a concentration typically used for red wine storage.

Even though AAB are generally regarded as aerobes, they are routinely isolated from wine samples taken from the bottom of tanks and barrels, where conditions would be considered to be anaerobic (Joyeux *et al*., 1984; Drysdale and Fleet, 1985). This suggests that they are able to survive and possibly grow under anaerobic to semi-anaerobic conditions in these environments (Drysdale and Fleet, 1989). The brief aeration of red wine during racking and transfer operations is sufficient to encourage the growth of AAB and cause wine spoilage, even when SO_2 has been added (Millet and Lonvaud-Funel, 1999). The spoilage of wine is not restricted to storage and maturation in a barrel or tank, but may also occur in the bottle. Bottles that are stored for extended periods of time in an upright, vertical position (several months), rather than in the horizontal position, are particularly prone to spoilage (Bartowsky and Henschke, 2004). This is because oxygen from the air somehow manages to enter the bottles in an upright position, when they are sealed with cork.

Wines in bottle with a visible spoilage are characterized by a distinctive circular deposit (ring) at interface of the wine and headspace or just below the closure and such visibly spoiled wines will have a spectrum of aroma and flavour defects,

including overt volatile acidity, loss of fruit aroma and oxidized or aldehyde character (Bartowsky and Henschke, 2004).

The presence of oxygen plays an important role in the growth of AAB in wine, as it is used as the terminal electron acceptor during respiration. Previous studies have shown that *Acetobacter* species are able to survive for quite extended periods of time in anaerobic conditions and, when exposed to small concentrations of oxygen, are able to proliferate (Joyeux *et al*., 1984). The control of oxygen is an essential tool in preventing wine spoilage by AAB. Careful winemaking practices include use of the correct dosage of SO_2 during wine maturation and filtering the wine prior to bottling, which can reduce the risk of wine spoilage by AAB in bottled wine.

The most significant endospore-forming bacteria in wine spoilage are the *Bacillus* and *Clostridium* spp. These bacteria mainly cause wine spoilage by increasing acidity (butyric acid) and sediment formation (microbial haze). Bartowsky and Henschke (2004) reported that “all is well until oxygen enters the scene” in relation to wine spoilage. Oxygen is associated with spoilage of wine by causing oxidation and favouring acetic acid bacteria and their growth in wine.

The presence of lactic acid bacteria, especially *Oenococcus oeni*, may be sometimes encouraged during vinification with grapes because of the positive flavour attributes conferred to the wine in comparison to the AAB species. Acetic acid bacteria are wholly undesirable in wine because they only endow the wine with negative attributes, particularly through the excessive production of acetic acid and ethyl acetate (Bartowsky and Henschke, 2004). The biochemical basis for the formation of acetic acid in wine is: ethanol $\longrightarrow$ acetaldehyde $\longrightarrow$ acetic acid.

The two membrane-bound enzymes that catalyse the reactions during the acetic acid formation in wine are: alcohol dehydrogenase, changing ethanol into acetaldehyde and acetaldehyde dehydrogenase changing acetaldehyde into acetic acid.

Although the production of vinegar in a wine bottle is not desired by the winemaker, it seems to happen quite often. Sensorially, acetic acid is recognised in wine as having a sour, vinegar-like aroma and flavour (Bartowsky and Henschke, 2004). The major volatile acid in wine is acetic acid (>90%) (Radler, 1993). Acetic acid has a threshold value of 0.7 to 1.1 g/L depending on the style of wine and above these values it becomes objectionable (Zoecklein *et al*., 1995). High levels of volatile acidity may not only originate from AAB and LAB, but also result from indigenous wine yeasts and

wild yeasts (for detailed review on spoilage of yeasts, see Du Toit and Pretorius , 2000).

The other measures that can be taken to control microbial spoilage in wine include appropriate levels of the hydrogen ion concentration (pH), alcohol, sorbic acid, fumaric acid, carbon dioxide and pressure, nitrogen availability and biological control (bacteriophages).

2.7 COMMERCIAL ENZYMES IN JUICE PROCESSING AND WINEMAKING

Enzymes play a definite role in the ancient and complex process of winemaking. From the pre-fermentation stage, through fermentation, post-fermentation and aging, enzymes are the major driving forces catalysing various biotransformation reactions (Van Rensburg and Pretorius, 2000). In fruit processing, enzymes have the task of hydrolysing the polysaccharides in the fruit, such as pectins, which make it difficult to extract juice from the mash or to clarify it. In order to prevent post-clouding (turbidity) in juices and concentrates, starch and araban need to be hydrolysed enzymatically (Schmitt, 1988).

2.7.1 Role of pectolytic enzymes

Pectolytic enzyme preparations have been used for over 60 years in fruit juice production (Oslen, 2000). These enzymes play a major role in fruit juice technologies, especially as prerequisites for obtaining well clarified and stable juices, and obtaining higher juice yields as well as high quality concentrates.

Pectinases, which are composed entirely of polygalacturonases, pectinlyases and pectin esterases, play a role in breaking down pectin chains. However, with more understanding of the complex pectin molecule, it has been realized that other enzymes, such as rhamnogalacturonases, xyloglucanases, arabinogalactanases, arabanases, etc., also play an important role in breaking pectin chains as reported by Gist-brocades (2000).

Pectin esterase (PE) is highly specific for the methyl ester of polygalacturonic acid. The pectin methylesterase (PME) splits the methyl group of polygalacturonic acid, proceeding in a linear fashion along the chain and thereby freeing methanol and converting pectin to pectate (McKay, 1988). PME is found naturally in bananas (Hultin and Levine, 1965). This means that methanol can also originate from the

action of PME naturally occurring in the banana fruit without addition of PME enzymes. The source of methanol in wine is pectin, which is hydrolyzed by PME enzymes that exist naturally in must. Addition of pectolytic enzymes to the wine in order to facilitate clarification, by breaking the 1-4 bond of the pectin polymer, also increases the methanol content (for a detailed review on methanol in must and wine composition, see Margalit,1997).

Esterases require at least one free carboxyl group adjacent to the methyl group under attack and are reported to attack the chain from the reducing end, transforming pectin to low methoxyl pectin, pectic acid and methanol (Van Rensburg and Pretorius, 2000). As regards the effect of enzymes on methanol levels in fermented products,it has been reported that the addition of pectolytic enzymes induces an increase of methanol levels in different fermented products, such as ciders (Massiot *et al*., 1994) and wine (Servili *et al*., 1992; Bosso, 1992; Bosso & Ponzetto, 1994). Nicolini *et al*., 1994, however pointed out that many other factors, such as grape variety, oenological practices and yeast strain used, can also influence methanol production.

Polygalacturonases (PG) break down glycosidic bonds that connect the molecules of galacturonic acid to one another, with the absorption of one molecule of water (Blanco *et al*., 1994). As polygalacturonases act on molecules with free carboxylic groups, they have little effect on highly methylated pectin in the absence of pectin methylesterases, and thus function synergistically with pectin methylesterases (Gainvors *et al*., 1994). The increase in the end groups is accompanied by a strong reduction in the viscosity of the substrate solution (Whitaker, 1990). Pectin lyase (PL) is particularly specific for highly esterified pectin, whereas pectates and low methyl pectins are the best substrates for endopectate lyase.

2.7.2 Juice extraction from the fruit

Fruit juice including banana juice can be extracted by enzymatic or mechanical means. However, the juices extracted by the two methods may differ in certain characteristics and composition (see Table 2.8).Different researchers have used different commercial enzymes(especially those with pectinolytic activities) in processing banana beverages (for detailed reviews on use of enzymes in banana beverages processing, see Viquez *et al*., 1981; Gous *et al*, 1987; Mabesa *et al*., 1989; Koffi *et al*., 1991; Kotecha *et al*., 1994; Shahadan & Abdullah, 1995;

Kyamuhangire *et al*., 2002; Jackson and Badrie, 2002). However, all the above mentioned researchers seem to have used only one particular banana cultivar in each study without broader comparison in physicochemical characteristics of juices and wines from different cultivars. In this study, physicochemical characteristics of banana juices and wines from three banana cultivars (including *Musa*, AAA genotype, traditionally utilized as dessert) were compared. Other researchers who have extracted banana juice mechanically for various purposes include Kundu *et al*. (1976), Akingbala *et al.* (1992), Sims *et al*. (1994), Gensi *et al.* (2000) and Kyamuhangire & Pehrson (1998, 1999). These researchers narrate stories regarding the challenges encountered in juice extraction and those challenges seem to be hinged on pectinaceous and oxidative nature of the banana pulp.

Pectin, which is a structural compound of the cell wall, is responsible for the firmness and colloidal nature of bananas. The low free-run juice yield and long pressing time therefore are due to the pectin level of the fruit. Breakdown by enzymatic pectin hydrolysis releases more free flow juice. This enables the formation of a press cake, from which more juices may be pressed (Pilnik, 1996).

Table 2.8. Yield,some characteristics and composition of banana juice extracted by enzymatic and mechanical methods

	Enzyme-extracted Juice	**Mechanically extracted juice**	**LSD p=0.05**
Pure juice yield (gkg^{-1})	604	541	3.8
Soluble solids(Brix)	34.9	30.7	1.3
pH	4.03	4.49	0.05
Titratable acidity(gkg^{-1})	5.4	3.3	0.26
Density (Mg m-3)	1.13	1.12	0.004
Viscosity($g\ m^{-1}s^{-1}$)	3.43	4.34	0.64
Nitrogen(gkg^{-1})	0.8	0.2	0.04
Sugars(gkg^{-1})			
Total sugar	259	253.3	1.78
Sucrose	1.3	165.2	1.01
Fructose	122.5	42.7	1.31
Glucose	130.7	44.8	0.75
Acids (gkg^{-1})			
Malic	6.1	5.9	0.44
Citric	3.7	3.8	0.38
Succinic	2.1	2.1	0.37
Minerals(gkg-1)			
Ash	10.1	12.2	2.8
Potassium	4.04	2.94	0.1
Magnesium	0.32	0.33	0.02
Phosphorus	0.23	0.24	0.03
Calcium	0.07	0.09	0.02
Sodium	0.03	0.02	0.01

Results are means of 20 experiments.

Source.Kyamuhangire and others (2002).

Experiments in which enzyme preparations (Vinozym ® Novo Nordisk Ferment) were added continuously to crushed grape mash at the rate of 2 g/hL showed the influence of the enzyme on free-run juice, with a juice yield of 93% compared to the control, with a yield of 63% (Villettaz, 1993). Munyanganizi and Coppens (1976) extracted juice from bananas by pectolytic treatment with an optimum yield (88%) at 0.05% enzyme dosage. Kotecha and Desai (1995) reported that the optimum enzyme concentration for obtaining juice from the pulp of the banana was found at 0.2% pectinase at 4 hr incubation period at 28 ± 2^{0}C. Enzymatic mash treatment in grape winemaking does not only influence juice extraction, but also the level of aromatic compounds such as terpenols.

The fermentation of juice containing too many suspended solids (resulting from juice extraction) does not produce quality dry wine. In fact; high concentrations of suspended solids in juice are known to have detrimental effects on wine quality. The suspended solids cause clarification difficulties. The first criterion of a juice extraction method, therefore is its ability to produce clear juice with a turbidity as near as possible to desired levels (Ribéreau-Gayon *et al*., 2000). Viquez *et al*. (1981) confirmed that pectinolytic enzyme treatment of banana pulp would enable a processor to obtain good yields of clear juice under practical conditions.

2.7.3 Liquefaction

Whenever fruit pulp is liquefied enzymatically (treated with pectinases and cellulases), pressing of the pulp may not be necessary. The liquefied juices are almost clear (papaya, cucumber), cloudy (apples, peaches) or pulpy (carrots), depending on the accessibility of the cell wall compounds to the enzymes. The liquefaction products can be clarified further by usual techniques; increased preference is given to ultrafiltration (Nagodawithana and Reed, 1993). Enzymatically liquefied banana pulp like the apples and peaches gives a cloudy juice at the bottom of the container with the lighter solid phase floating on top. Normally, low viscosity values after enzymatic treatment correspond to complete liquefaction. Liquefaction is obtained by the enzymatic breakdown of cell wall polysaccharides to low molecular water-soluble carbohydrate constitutes. Microscopy of the pulp before and after liquefaction indicated that no cell walls were left in the liquefied juice. Enzymatic liquefaction has proved to be suitable for both small- and large-scale juice processing

operations (Pilnik, 1996). Kyamuhangire *et al*. (2002) extracted banana juice using enzymatic and mechanical methods. They reported that the average viscosity of banana juice extracted by the mechanical method was higher than that of juice extracted by the enzymatic method.

2.7.4 Maceration

Maceration refers to a process by which organised tissues are transformed into a suspension of intact cells, resulting in pulpy products, usually used as base material for pulpy juices and nectars, as baby food, and as ingredients for diary products such as yoghurt. Since the aim of the enzyme treatment is the transformation of tissue into a suspension of intact cells (Grampp, 1972; Bock *et al*., 1983), pectin degradation should affect only the middle lamella pectin. This is the process called maceration. The so-called macerates are enzyme preparations that degrade pectin in fruits and are reported to contain only polygalacturonase (Zatelaki-Horath and Vas, 1980) or pectin-lyase activity (Ishii and Yokotsuka, 1971; 1973). Mabesa *et al*. (1989) noted that banana juice was more easily to press in the enzyme treated pulp as compared to the untreated pulp. And they suggested that this could be the result of the decrease in the viscosity of the juice in the pulp due to the solubilisation of the pectin. The inactivation of endogenous pectin esterase is vital for the maceration of many products (Dongowski and Bock, 1984). Most uses of exogenous pectinases for fruit juice extraction and clarification are based on the destruction of pectin and other desirable fibre components of the fruits (Nagodawithana and Reed,1993).

2.7.5 Juice yield

Generally, banana pulp has been reported to be too pectinaceous to yield juice by simple pressing or centrifugation (Viquez *et al*., 1981). The extraction of juice from bananas is a physical process, whereby the banana pulp is mixed with extraction aid materials such as spear grass (*Imperata cylindrica*) and polythene strips (Kyamuhangire, 1990; Aked and Kyamuhangire, 1996) to enable manual workability of the pulp to yield juice. The yield of pure juice has been reported to be as low as 38.1% and as high as 60 to 65% in non-enzymatic extraction and pectolytic enzyme-influenced extraction respectively (Viquez *et al*., 1981; Sims *et al*., 1995). However, it has been reported that dilution of the juice will not affect the quality of the wine, as

long as the dilution is done to acceptable levels. Therefore, some farmers use water to extract juice from spent pulp (pomace) and to increase yields. Juice extracted from banana (*Musa* AAAcv, Giant Cavendish) by Viquez *et al*. (1981) in percentages weight by weight is presented in Table 2.9. The various enzymes used influenced juice yields in different percentages. However, it is very clear that the role played by the enzymes in juice extraction was very important in comparison with the control. Such enzymatic action in juice extraction has been explored for long and still requires mores studies to establish appropriate conditions and enzyme dosages in fruit pulps for beverage production operations.

Table 2.9:Average juice yields obtained with banana of different grades of ripeness (1-3) using different enzymes

	Average juice yield(%w/w) at different grades of ripeness		
Enzyme	**Grade 1**	**Grade 2**	**Grade 3**
Pectinol	49.6	53.3	57.9
Pectinol D	50.2	54.1	58.4
Pectinase PV8	46.9	46.9	51.4
Ultrazym 100	50.0	52.5	58.0
Ultrazym 100 special	66.5	68.4	68.8
Clarytine Super	66.4	67.4	68.8
Control	4.8	20.7	27.5

The figures are averages of three repetitions.
The control data refer to juice yield following incubation in the absence of added enzyme
Source:Viquez et al., (1981).

2.7.6 Aroma extraction

Aroma is volatile and it is an essential pre-requisite for perception of a pleasant flavour in a food product. For efficient extraction of aroma, red wine is fermented on the grape skins. Whereas flavour refers to the effects of both odour and taste, aroma is purely associated with odorous, volatile compounds, while the bouquet of wine refers to the more complex flavour compounds which evolve as a result of fermentation and aging (for a detailed review on wine aroma, see Lambrechts and Pretorius,2000). The floral aroma of grapes and other fruits was found to be caused mainly by a group of substances named monoterpenes (Margalit, 1997). The fruit monoterpenes precursors of terpenols are the bonded terpenes and the polyols. Monoterpene glycoside precursors are non-volatile and therefore without any significant aroma. The quantity of these precursors can be higher than the amount of

aromatic terpenol, indicating increased flavour potential (Dimitriadis *et al*., 1985). Linalool and geraniol are two of the most abundant bound terpenols (Gunata *et al*., 1985; Ribereau-Gayon *et al*., 1975). These particular two terpenols are the most aromatic, meaning that they have a very low olfactory threshold (Gunata *et al*., 1985, Marais, 1983; Ribereau-Gayon *et al*., 1975). In aromatic grapes, terpenols in various states of oxidation form the major part of the aroma (Williams *et al*., 1980, Williams and May, 1981). Terpenols have been known to interact synergistically with one component, increasing the aroma of another component (Marais, 1983). Bonded terpenes can undergo both acid- or enzyme-catalysed hydrolysis to provide volatile aroma compounds (Williams *et al*., 1980). The main groups of compounds that form the "fermentative bouquet" or "fermentative aroma" are the organic acids, higher alcohols and esters, and to lesser extent aldehydes (Rapp & Versini, 1991). The most "negative" aroma compounds are the reduced sulphur compounds, hydrogen sulphide, organic sulphides and thiols.

Fermentative aroma is not only brought about by the conversion of directly fermantable substances, but also by the long-chain fatty acids, organic nitrogen-containing compounds, sulphur-containing compounds and many others. These compounds are able to penetrate from the grape juice medium through the yeast cell wall membrane, where they participate in biochemical reactions producing numerous volatile substances as by-products (Boulton *et al*., 1995). Some of the esters produced by yeast which were reported (Salo, 1972; Riesen, 1992; Boulton *et al*., 1995) in bananas include isoamyl acetate, isobutyl acetate and ethyl hexanoate.

2.7.7 Juice and wine clarification

Freshly extracted fruit juices are more or less turbid. They contain suspended solids of diverse origin that may include earth, skin, stem and cellular debris from the fruit. Clarity is an essential quality required by consumers, especially for white wines in clear glass bottles. Particles in suspension, either in forming a haze or dispersed through the liquid, not only spoil the presentation but usually also affect flavour. New wines have very high particle content, consisting of yeast lees and other grape debris. Clarity is achieved by gradual settling, followed by racking to eliminate the solids. Other more rapid processes (filtration and centrifugation) may be used (for a detailed review on clarity and stability of juices and wines, see Ribéreau-Gayon *et al*.,

2000). Earlier on, Beltman and Pilnik (1971) had confirmed that enzymatic pectin degradation yields thin free-run juice and a pulp with good pressing characteristics. Fruit juice clarification is the oldest and still the largest use for pectinases, which are applied mainly to deciduous fruit juices and grape juice (Kertesz, 1987). The traditional way of processing juices is by crushing and pressing the pulp. The raw press juice is a viscous liquid with a persistent cloud of cell wall fragments and complexes of such fragments with cytoplasmic protein (Nagodawithana and Reed, 1993). Addition of pectinases to cloudy raw press juice lowers the viscosity and causes cloud particles to aggregate to larger units (flocks), which sediment and can easily be removed by centrifugation or (ultra) filtration (Sims and Bates, 1994).

Wines made from clarified juices are easier to clarify (Amerine *et al*., 1980). Clarification involves physical means of removing suspended particulate matter from the juice or wine. Pectinases are used for clarification in order to obtain a limpid product. When observing clarity, it may be easily seen by turbidity of the product. This may not be easily seen with a naked eye but rather with the help of an instrument like the nephelometer in nephelometric turbidity units (NTU).

Turbidity in wine is due to the presence of particles in suspension that stop light rays and diffuse some of the light in other directions than that of the incident beam and this makes the wine seem opaque to varying degrees (Ribéreau-Gayon *et al*., 2000). Like starch, araban, which consists exclusively of arabinose, may be the cause of post-clouding in juices and wines. During the post-extraction of pomace, araban sometimes gets into press juice and may not be easily eliminated by clarifying agents and precipitates during storage of the juice or wine. In normal conditions, juice turbidity generally decreases during grape maturation (Hadjinicolaou, 1981). This evolution results from the hydrolysis of pectic substances in the berry by pectic enzymes of the grape *viz*: endopolygalacturonase and pectin esterase (Ribéreau-Gayon *et al*., 2000). Therefore, addition of exogenous enzymes in the form of commercial preparations supplements the endogenous enzymes activities. The role of pectinases is illustrated in the flow diagram of fruit juice manufacture in Figure 2.2 by Pilnik and Voragen (1989).

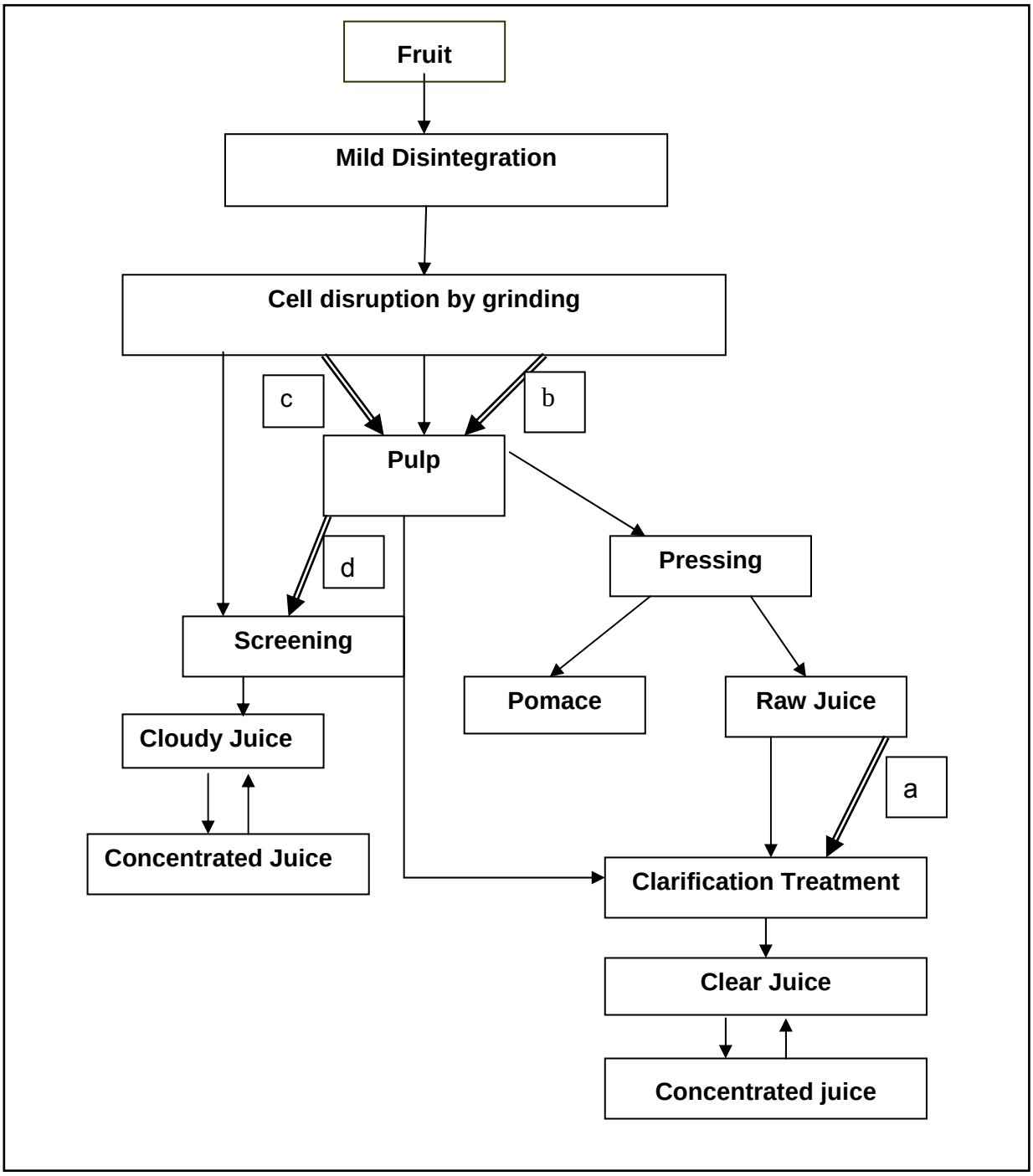

Figure 2.2: Flow diagram of fruit juice manufacture.

In the Figure 2.2 above, the arrows indicate eventual enzyme treatments by (a) pectinases for clarification; (b) pectinases for pulp degradation; (c) pectinases and cellulases for liquefaction; and (d) polygalacturonase, pectin lyase, or pectate lyase for maceration.

2.7.8 Juice filterability

Filterability is defined as the number of millilitres of juice obtained from 100 g of puree following vacuum filtration (10 *psi*) for 2.5 minutes through Whatman filter paper. Due to the fact that pectinases are used to reduce the viscosity of grape must through the hydrolysis of pectin, the benefits of these pectinases include enhanced filterability, improved free-run juice yields, improved juice settling rates and clarification (Berg, 1959). Previous research on bananas has reported that ripe bananas contain approximately 3-4% total fibre (Paul and Southgate, 1978), with about 1% cellulose (Southgate, 1969), 0.5-1% pectin (Kawabata and Sawayama, 1974), and 1-2% hemicellulose (Berell, 1943). Ripe banana also may contain 1-4% starch (United Fruit Co., 1961). Pectinase, cellulase, hemicellulase and amylase are able to reduce viscosity and increase filterability of banana puree as earlier research had reported (Koffi *et al*., 1991).

2.8 FOOD SAFETY ASPECTS

Whereas for any food manufactured quality is nutritionally required, the food safety must be guaranteed to the consumer. Food legislations and regulations are always in place to protect the consumers and penalise those who produce foods that are below the required standards. Standards of identity for beers, wines and spirits beverages stipulate the need for analyses such as percentage alcohol by volume, total solids content, volatile acidity and calculated acidity (Nielsen, 2003). There are competent bodies established with special technical staff or committees to ensure food safety and quality and these include ISO, WTO, TBT, WHO, FAO, CAC and NBS (see Acronyms, Appendix 1). In food production and distribution chains, unsafe food may be a result of deliberate (food adulteration and use of food additives that are not GRAS) and none deliberate (food poisoning by contaminated agents especially microbes) changes that render a food unsafe. Ensuring food safety must be a responsibility of all people processing, manufacturing or handling food at all levels.

However, it has been ascertained (West *et al*., 2006) that assessing the risk for hypersensitivity to novel whole foods is difficult. Smith (2001) stated that safety assessment of novel foods and food ingredients must satisfy the producer, the manufacturer, the legislator and the consumer. He further added that the approach

should be in line with accepted scientific considerations, whereby the results of safety assessment must be reproducible and acceptable to the health authorities and the outcome must satisfy and convince the consumer. The risk assessment of GMO product (including GM foods) have been made by experts and judged on the basis of safety to the consumer. All experimental release (GMOs) trials must have government approval and the applicant provide detailed assessment of the risk of harm to human health and/or the environment.

The safety of GMOs continues to be addressed by scientific research. Basic research into the nature of genes, how they work and how they can be transferred between organisms has served to underpin the development of the technology of genetic modification. In this way, basic information about the behaviour of genes and GMOs will be built up and used to address the concerns about the overall safety of GMOs, GM foods and their impact on the environment (for a detailed review on safety of genetically engineered foods, see Smith, 2001).

In agreement with the above views, it requires scientific accuracy and precision of technical committees to certify research results for authenticity of novel foods for human consumption. For experimental purposes on recombinant yeast used in our study (Chapter 5), the above mentioned observations were considered and adhered to in the protocols used in experiments.

In Uganda, where banana raw material is in abundance for various product processing (including biotechnological use of native yeasts to ferment a common beverage-*tonto*), tremendous work has been done regarding Biotechnology and Bio safety. The Biotechnology and Bio- safety Policy was developed with a vision to make Uganda a country safely utilizing biotechnology as a tool for national sustainable development in the context of Poverty Eradication Action Plan (PEAP), Vision 2025 and the Millennium Development Goals among others (Uganda National Council for Science and Technology, 2006).

Uganda National Council for Science and Technology (2006), further reports that government developed the Biotechnology and Bio safety Policy in line with the Cartagena Protocol on Bio safety to the Convention on Biological Diversity (CBD) and the National Science and Technology Policy of 2001 that provides for the

formulation of a biotechnology policy to guide the judicious use of biotechnology for sustainable development.

Globally, there are different initiatives focusing on modern biotechnology development, adoption, safe use, benefit sharing and trade. These lead to the development and negotiation of the Cartagena Protocol on Bio safety under the United Nations Convention on Biological Diversity (Uganda National Council for Science and Technology, 2006).

However, FAO / WHO (2002), through Codex Alimentarius Commission (CAC) published that there is growing international concern related to a perceived emergence of or increase in food-borne diseases. Consumers around the world are more aware than ever about food safety issues and seeking ever –greater assurances about the safety and quality of the foods they eat. Innovation and development of new processes (including modern biotechnology) are leading to the development of new products with specific medicinal, nutritional and functional attributes. In its endeavour to promote food safety and quality, the CAC needs to consider opportunities for strengthening partnership with all stakeholders, in particular consumers and their representative organisations at the global and national levels. The CAC does not undertake scientific evaluations per se but relies on the opinions of scientific expert committees or consultations convened by FAO and WHO on specific issues. These expert bodies (FAO/WHO) have expert committees of concern on food additives, pesticide residues and microbiological risk assessments to ensure food safety.

Aware that fermented beverages can be a source of food poisoning, control measures against contamination, especially by microorganisms are always practiced. Uncontrolled microbial growth before, during or after wine fermentation can alter the chemical composition of the product, detracting it from its sensory properties of appearance, aroma and flavour. Healthy fruits, cellar hygiene and sound oenological practices are the cornerstones of the wine maker's strategy against the uncontrolled proliferation of spoilage microbes. Added safety is provided by the addition of chemical preservatives, such as sulphur dioxide, dimethyl dicarbornate, benzoic acid, fumaric acid and sorbic acid which control the growth of unwanted microbial contaminants (Du Toit and Pretorius, 2000).

This particular research study on banana juice and wine followed the same techniques previously used by above researchers in accordance with GMP and the chemical additives (DAP, SO_2, citric acid) used are among the GRAS and have been used the in manufacture of various conventional foods. And in consideration of the above views on food product safety, recommendations for future use of biotechnological processes in the banana juice and wine are made based on our results and with a view that there will be the necessary scientific analyses to monitor the required food safety standards.

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CHAPTER 3
RESEARCH RESULTS: CHARACTERISTICS OF ENZYME-TREATED BANANA JUICE FROM THREE CULTIVARS OF TROPICAL AND SUBTROPICAL AFRICA

Abstract

Enzyme treated banana juices of three cultivars from tropical and subtropical Africa were studied. The main aim in this study was to apply commercial enzymes to banana pulp and improve on juice extraction and clarification. The banana cultivars which were selected for this study were *Kayinja* (ABB genotype), *Mbidde* (AAA-EA genotype), and *Bogoya* (AAA genotype). Two types of banana belonging to the same genotype (*Musa*, AAA) grown in the tropical and sub-tropical regions of Africa, known as *Williams* and *Gros Michel* in South Africa and Uganda respectively were used for comparison of physicochemical properties. All the cultivars were purchased at full maturity and ripened to stage 8 (yellow, speckled brown) of ripeness under similar warm conditions. Different commercial enzyme preparations were added to banana pulps in the preliminary experiments at the rate of 0.03 g/L for pectin de-pectinisation and de-esterification at 15°C for 24 hours. After juice extraction four pectinolytic enzymes (*Rapidase X-Press, Rapidase CB, Rapidase TF* and *OE*-Lallzyme) were selected for further experiments. The parameters that were analysed for comparison included juice yield, total soluble solids, total acidity, pH, viscosity, specific gravity and turbidity. The juice yield was significantly improved (477% v/w) in one of the enzyme treated samples compared to the control in the Kayinja (ABB genotype). The soluble solids in the juice of the three cultivars ranged between 15^{o} and 27^{o}Brix. The total soluble solids of bananas of the same genotype but grown in different climates differed significantly (p<0.05). Turbidity was lower in all the enzyme-treated juices samples compared to the control samples in the 3 cultivars. The overall acceptability of the juices was assessed by a panel and scored more than six points on the nine-point hedonic scale that is at least 66.7% of the juice is acceptability in general.

Keywords: Enzymes, banana juice, *Musa* genotypes, juice yields and clarification

3.1 INTRODUCTION

Banana (*Musa* spp.) juice is nutritionally an important beverage in the tropical and subtropical world and is rich in calories and minerals such as potassium, magnesium, phosphorus, etc (Kyamuhangire *et al*., 2002). With proper food hygiene under Good Manufacturing Practices (GMP), safe and quality banana juice can be processed from over-ripe rejected fruits. Bananas rejected on market because of external injury or over supply are potential sources of raw material for juice processing. Clear banana juice, with its widely appreciated flavour and aroma can be used in various foods and drinks (Shahadan and Abdullah, 1995).

Unfortunately, there is considerable waste of the “rejected bananas”, i.e. those that do not meet the quality norms for export (Viquez *et al*., 1981). This is due to the fact that the banana fruit is biologically active and carries out transpiration, ripening and other biochemical activities even after harvests, which deteriorate the quality of the fruit and finally make it unmarketable (Emerald and Sreenarayanan, 1999). Wastage of the banana fruit due to poor post-harvest handling or over-ripening remains a major problem today (Shahadan and Abdullah, 1995). Fruit losses can be reduced by processing over-ripe fruits into other products such as wine and vinegar (Adams, 1978). However, some tropical fruits, banana inclusive, are too pulpy and pertinacious to yield juices by mechanical methods without the expenditure of excessive amounts of energy (Kyamuhangire *et al*., 2002; Shahadan and Abdullah, 1995).

Banana beverages in the form of juice and traditional beer have been produced locally for several years in the banana-producing areas. Though not yet commonly available on the market, clarified banana juice has been studied by different researchers using various approaches (Viquez *et al.*, 1981; Koffi *et al.*, 1991).

Of the problems associated with banana juice processing, a high viscosity, problems with juice extraction (Dupaine and Delnic, 1965; Viquez *et al*.,1981) and browning problems (Galeazzi and Sgarbieri, 1981; Mao, 1974) seem to be the most severe (Sims and Bates, 1994). Sulphite and other chemicals have shown to inhibit banana polyphenoloxidase (Koffi *et al*., 1991) although safety concerns may limit its use (Sims *et al*., 1994). The use of commercial enzyme preparations in the fruit juice

processing industry to facilitate juice release and increase juice yield is well established (Kyamuhangire *et al*., 2002).

The use of commercial pectinolytic enzymes as processing aids has been done with various fruits such as apple and grape to disintegrate the fruit pulps, reduce viscosity and clarify the juices (Roumbouts and Pilnik, 1978). The action of enzymes leads to degradation of and volatilisation of other insoluble materials (pectin, hemicellulose and some cellulosic materials) from the fruit pulp cell wall, resulting in increased juice yield (Kyamuhangire *et al*., 2002). The juice is subjected to enzyme treatment to produce the clear juice (Waldt and Mahoney, 1967). This enzymatic treatment of fruit juices has proved to be a reliable biotechnological means of producing limpid juices. Therefore, there is a need for the production of value-added products and for market development strategies (Mazk and Degner, 1994).

In this study, we investigated the effect of commercial enzymes on juice yields, clarification, total soluble solids, titratable acidity, pH, viscosity and organoleptic characteristics on juices from three different banana cultivars grown in tropical and subtropical Africa. Specific comparison of juices from 2 types of banana called *Gros Michel* in tropical Uganda and *Williams* in subtropical South Africa were made. This was chosen for comparison of physicochemical properties because the two types belong to the same genotype (*Musa* AAA, genotype) and raw materials for experiments were easily accessible in the two countries where the study was conducted.

3.2 MATERIALS AND METHODS

3.2.1 Banana cultivars used and pulp preparation

Three banana cultivars were used to study banana juices and wines. Kayinja (ABB, genotype), Mbidde (AAA-EA, genotype) and Bogoya (AAA, genotype) bananas were used in this study. Kayinja and Mbidde cultivars were purchased at the mature stage 1 of ripeness from local farmers in the Wakiso district in Uganda. ”Williams” as it known in South Africa, commonly known as “*Gros Michel*” or Bogoya in Uganda, at stage 3 of its ripeness was always purchased from “Fruit and Veg. City” supermarket in Stellenbosch, South Africa. The bananas bought in Uganda were exported to

South Africa for experiments at the Institute of Wine Biotechnology, University of Stellenbosch. The bananas were always ripened to stage 8 (yellow speckled brown) under special warm conditions for seven to ten days at a temperature of between 28 and 33°C. This stage of ripeness before processing was desired because it is similar to the stage of the usually wasted ("rejected") bananas and the major issue was to see whether safe, stable and quality beverages can be obtained in over-ripe form. The ripened bananas were peeled manually and pulped using a motorized mixer before the pectinolytic commercial enzymes were added. Three banana pulps were weighed and separated in equal portions in their respective cultivar groupings. Then banana pulp were treated with commercial enzymes at recommended dosages and left for 24 hour reaction time before juice extraction.

3.2.2 Enzymes used

Various pectinolytic enzymes were purchased and used to treat the banana pulps during the preliminary experiments. However, the four enzymes that were chosen for subsequent experiments were selected based on their capacity to produce higher juice yields than the rest of the enzymes. The concentration of the enzymes that were selected after being applied as recommended by the supplier is shown in Table 3.1. The enzymes that were not chosen for further studies and the yields obtained from banana pulps are shown in Table 3.2. All the commercial enzymes were applied in three experiments in duplicates at 15^0C.

Table 3.1: The four enzymes that were selected after preliminary juice extraction.

Enzyme	Description	Dose	Producer
Rapidase X-press	Pectinase activity	0.03 g/kg	Gist-brocades
Rapidase CB	Pectinases, maltodextrine, NaCl	0.03 g/kg	Gist-brocades
Rapidase TF	Pectinase, hemicellulase, cellulase activity	0.03 g/kg	Gist-brocades
OE-Lallzyme	Pectinase activity	0.03 g/kg	Lallemand

The flow chart in Figure 3.1, below, illustrates the method used for processing banana juice from commercial enzyme-treated banana pulp in this study.

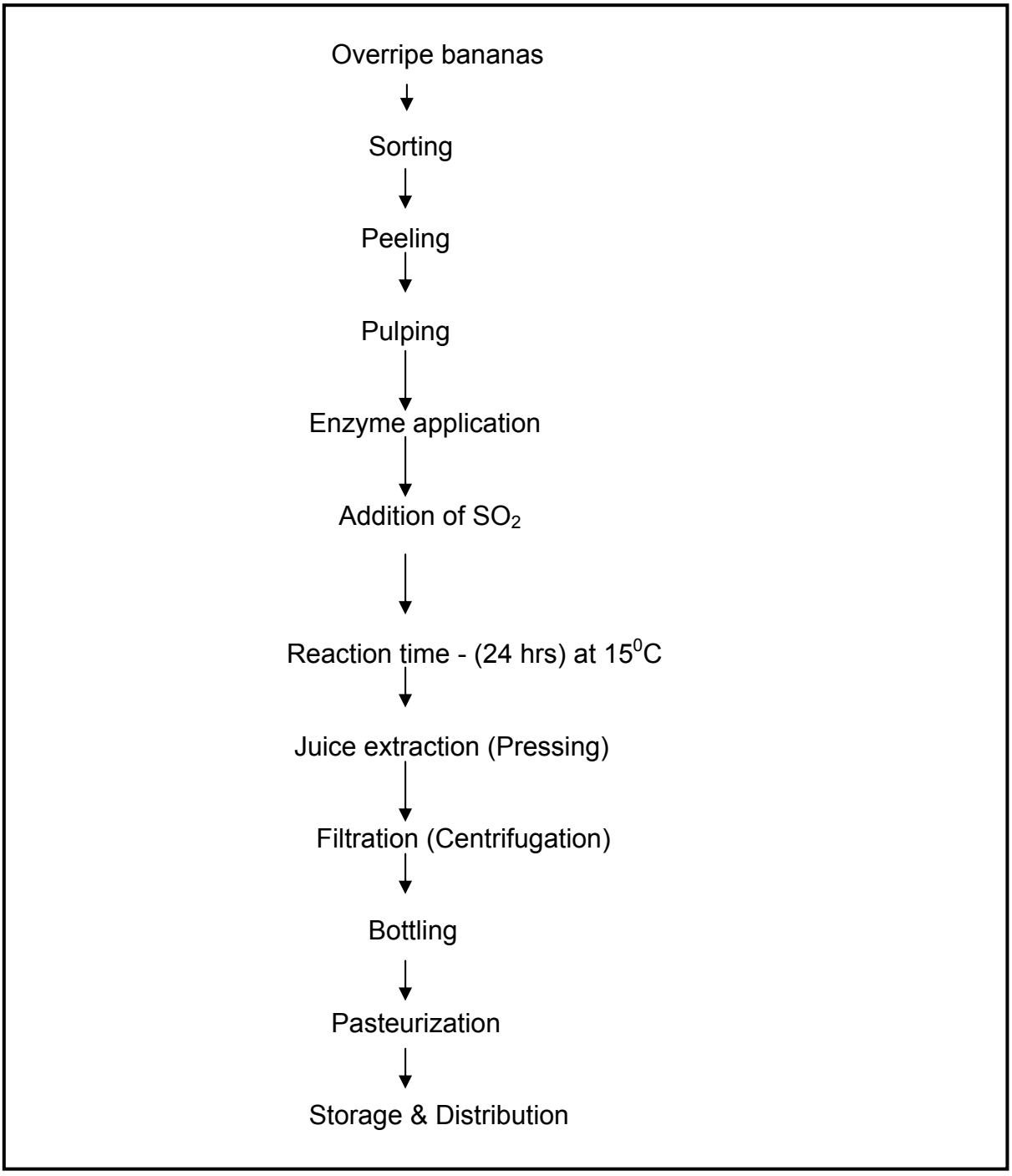

Figure 3.1: Flow chart for banana juice processing technology.

3.2.3 Juice extraction

Juice extraction was done by treating banana pulp with pectinolytic commercial enzyme preparations and leaving the untreated (control) sample for comparison. We started this study by doing a preliminary screening of available enzymes. Juice extraction at the preliminary stage was done in three experiments in duplicates. The main strategy was to choose enzymes that would influence higher banana juice yields than the others.

Bananas were peeled manually and pulped using a blender into a rough pulp. After the pulping, the pulp was divided in equal portions for enzyme treatment reserving portions to serve as control in each cultivar. Sulphur dioxide (SO_2), an antioxidant and antimicrobial agent was applied to all pulps at a concentration of 50 ppm. Enzymes were added and the pulps were left to stand for 24 hours at 15^0C. After 24 hours, free-run juice was siphoned and measured in millilitres. The pulps were pressed, using a basket screw-press to maximise juice yields and the juices were strained through cheesecloth. Juice yield determination was presented as a ratio of juice (litres) divided by mass (kilogram) of pulp and expressed in percentages volume by weight (%v/w). All the juices extracted were compared in terms of amount yielded as influenced by the various enzymes added. The strained juice was filtered using a pressure filter in sterile conditions and bottled. Some juices were bottled and pasteurized at 85^0C for 5 minutes before storage. Juice samples were collected for analyses and sensory evaluation. The rest of juices obtained using the four selected enzymes were used further for banana winemaking experiments.

3.2.4 Physicochemical analysis

The extracted juice samples were analysed for various parameters. A refractometer (ATAGO Co, Tokyo Japan) was used to determine the juice total soluble solids in °Brix. Titratable acidity and pH were automatically determined with the use of a 20-702 SM Titrino and with a glass calomel electrode pH meter(Cole Parmer, Vernon Hills ,IL, USA) respectively. Viscosity was measured, using viscometer Haake VT-02, in centipoises (cP). The specific gravity was determined with the use of a hydrometer at 20^0C. The turbidity of the juice was measured by a Hack turbidimeter in nephelometric turbidity units (NTU). The turbidity of the juices was determined in freshly extracted juices from both enzyme-treated and untreated banana pulps.

3.2.5 Statistical analysis

Analysis of variance (ANOVA) was performed on all juice yields and turbidity results (Zar, 1984). ANOVA was also performed on the viscosity of the juice. This was done using the STATISTICA 7 processing package.

3.2.6 Sensory analysis

Sensory evaluation was conducted at the Food Processing Laboratory, Department of Food Processing Technology, at Kyambogo University. Before the sensory evaluation, the panellists were subjected to a training session to familiarise them with the sensory attributes of the banana juice to be assessed. Each judge was introduced to a sensory evaluation score card and briefed on the procedures of evaluation during the training. A ready-to-drink (RTD) banana juice was presented as reference. To check on the assessor's consistency, a replicate of a juice was presented. A nine-point rating system was used. Quality evaluation was conduced by 20 trained and untrained teaching staff and students respectively. These could be considered as banana beverage connoisseurs because of their general knowledge, interest in and experience of local banana beverages. Samples were evaluated at room temperature of 25°C ±1°C under red fluorescent light and evaluation tests took place between 09:00 pm and 11:00 am. For each test, the panellist was served with 50 ml of banana juice in 100 ml colourless wine glasses. Coded juice samples that had been chilled at 7°C for 24 hours were served. Panellists were instructed to sip water and rinse their mouths between each of the juice samples provided.

The sensory attributes assessed by the panel of tasters were ranked from low to high levels on a nine–point hedonic scale. Taste was measured in terms of sweetness, whereas flavour and aroma were related to the banana flavour. The mouthfeel was assessed in terms of smoothness of the juice on the tongue. Acidity was assessed in terms of sourness of the juice in the mouth, a typical characteristic of high acetic acid. Colour of the juice was evaluated in terms of its intensity. Then, the overall acceptance was the general preference expressed by the assessors after evaluating the attributes in the sensory profile. The scores were subjected to statistical analysis at the Centre for Statistical Consultation of Stellenbosch University. To determine if

there was any significant difference between the banana juices produced by application of different enzymes and the control, repeated measures of ANOVA were performed with the Bonferroni multiple testing procedure.

3.3 RESULTS AND DISCUSSION

3.3.1 Juice yield

The results of the banana juice yields are presented in Table 3.2 and Table 3.3. The juice yields obtained from pulps with the enzymes added in Table 3.2 were lower than those obtained in Table 3.3. The highest increase in juice yield was obtained from Kayinja (*Musa*, ABB genotype) pulp, which was treated with pectinolytic enzyme Rapidase X-press. The Kayinja juice yield of 57.87% v/w which was extracted from Rapidase X-press enzyme treated pulp was 477% higher than that extracted from the control sample (pulp with no commercial enzyme added) that yielded 10.03%v/w. The four selected enzymes played a significant (p<0.05) role in influencing extraction of banana juice. This can be deduced from the percentage of juice increases obtained. Comparing the results from all three cultivars, it can be seen that the added enzymes were most effective on juice extraction from the Kayinja cultivar. This implies that the fruit pulp of Kayinja was degraded by the pectinolytic enzymes more effectively than Bogoya and Mbidde cultivars. The huge difference in increases in yields of control and the enzyme treated samples can be attributed to the firm nature of the Kayinja fruit. Its firmness can be felt physically when the over-ripe banana fingers are pressed and that character seems too be associated with the viscosity observed in pulp (caused by the polysaccharides and proteins). This firmness of Kayinja in an over-ripe state proved to be efficiently degraded by the pectinolytic enzymes and enable free-run juice release according to results of this study. Considering Bogoya and Mbidde, the highest juice yields obtained were also from the pulps treated with the enzyme Rapidase X-press, yielding 67.9% v/w and 77.9% v/w respectively. These results of fruit juice yield increases, influenced by commercial enzyme preparations with pectinolytic activities are in agreement with other research findings in various fruits and are associated with viscosity reduction in the pulp. Previous researchers (Mabesa *et al.,* 1989; Kyamuhangire *et al*., 2002; Fundira *et al*., 2002) had reported a similar phenomenon after adding commercial enzyme preparations to various fruit mashes at different temperatures and enzyme dosages.

This juice yield is mainly influenced by the de-polymerisation and de-esterification effect on the complex pectin molecules, degrading cell walls of pectinaceous nature and thus enabling easy release of fruit juice. The enzymes needed for pulp liquefaction are pectinases and exo-ß-glucanases (C1-cellulase, cellobiohydrolase). Exo-ß-glucanase works in conjunction with endo- ß-glucanase (C1-cellulase) which is present as *Aspergillus*-derived enzymes in most preparations (Nagodawithana and Reed, 1993). In our study, enzyme treatment of banana pulp gave cloudy juice. The enzymatic action leads to degradation and solubilisation of otherwise insoluble materials (pectin, hemicelluloses and some cellulosic materials) from the fruit pulp cell wall, resulting in increased juice yield (Dorreich, 1993). Because complete pectin hydrolysis is a prerequisite for successful clarification, the pectinases used in clarification contain especially high proportions of polygalacturonases and pectinesterases (Krug, 1968). The control samples from the three banana cultivars showed significantly (p<0.05) low juice yields, especially in Kayinja as compared to the other two cultivars. Such huge differences may be attributed to the genetic make up of the three banana cultivars with different genotypes and their natural capacity to yield juice with influence of endogenous enzymes in each cultivar especially Kayinja pulp which to a large part tended to remain colloidal (sort of slimy) not freely separating liquid-solid phases. It may also be related to high (24-27^{0}Brix) TSS in Kayinja which makes juice extraction in this particular cultivar more difficult than in the other used cultivars. Viquez *et al.* (1981) investigating juice yields from banana cultivar also found huge differences between control and enzyme treated juice yields. In their study, they found that the juice yield in the control (no enzyme addition) at banana grade 1 (beginning to ripen) of ripeness was 4.8% (w/w) compare to a yield of 66.5% (w/w) when the pectolytic enzyme Ultrazym 100 was added. They also tested the juice yield at grades 2 and 3 (medium ripe and very ripe respectively) of ripeness. The same tendency as the grade 1 ripeness was observed.

Experiments in which enzyme preparations (Vinozym ® Novo Nordisk Ferment) were added continuously to grape crushed mash at the rate of 2 g/hL showed the great influence of the enzyme on free-run juice, with a juice yield of 93% compared to the control, with a yield of 63% (Villettaz, 1993). Such a yield increase percentage (47.6%) of free-run juice is large and really worth enzymatic treatment of fruit mash.

Table 3.2: Juice yields influenced by enzymes excluded from further study.

Cultivar studied	Enzyme applied	Mean(%v/w)
Bogoya (*Musa*, genotype AAA)	Control	52.47
	EXV Lallzyme	52.47
	Rapidase filtration	52.67
	Rapidase pineapple	52. 62
	Hazyme	52.10
	Bats Liq	52.73
Mbidde (*Musa*, genotype AAA-EA)	Control	57.20
	EXV Lallzyme	56.86
	Rapidase filtration	57.37
	Rapidase pineapple	56.83
	Hazyme	57.80
	Bats Liq	57.27
Kayinja (*Musa,* genotype ABB)	Control	10.03
	EXV Lallzyme	36.87
	Rapidase filtration	35.67
	Rapidase pineapple	37.87
	Hazyme	36.23
	Bats Liq	39.47

Data are means of three experiments replicated

Table 3.3: Juice yields influenced by enzymes selected for further study.

Cultivar	Enzyme applied	Yield(%v/w)
Bogoya (*Musa* genotype AAA)	Rap. X-press	67.93 ± 1.27^{a}
	Rap. CB	56.73 ± 1.00^{bc}
	OE-Lallzyme	59.17 ± 0.61^{c}
	Rap. TF	64.19 ± 0.90^{d}
	Control	52.47 ± 0.71^{e}
Mbidde (*Musa* genotype AAA-EA)	Rap. X-press	77.93 ± 0.72^{ad}
	Rap. CB	74.43 ± 0.67^{b}
	OE-Lallzyme	63.07 ± 0.65^{c}
	Rap. TF	77.07 ± 0.38^{d}
	Control	57.20 ± 0.90^{e}
Kayinja (*Musa* genotype ABB)	Rap. X-press	57.87 ± 1.43^{a}
	Rap. CB	52.27 ± 0.97^{bcd}
	OE-Lallzyme	49.60 ± 0.82^{cd}
	Rap. TF	51.17 ± 1.31^{d}
	Control	10.03 ± 0.40^{e}

Values are means ± SD of three experiments replicated.
[a] Different letters in the same column indicate significant difference at $p<0.05$.

The results of this study in the three banana cultivars concur with those other results previously reported (though those seem to be only on one cultivar) and confirm the significant role played by pectinolytic enzymes in influencing banana juice yields. As an innovation to discover whether Bogoya (a traditionally known as dessert cultivar) could yield juice, commercial enzymes that were added to its pulp influenced substantial amount of juice yield from the over-ripe fruits. The untreated mashed

(pulped) Bogoya also yielded (an average of 52.5%) juice. This may create an alternative way of utilizing over-ripe Bogoya (as a beverage source) which traditionally was used as dessert and would be wasted as spoiled fruit in an over-ripe form. According to the findings in this study and those of other previous researchers (Kyamuhangire *et al*., 2002) enzyme treatment of banana pulp may be the best way to adopt for viscosity reduction and best way of juice extraction for banana beverages processing at large scale.

3.3.2 Physicochemical characteristics of juices obtained from three banana cultivars

The results of physicochemical characteristics of juices obtained from three banana cultivars are presented in Table 3.4 (a) and (b). For a comparative study of bananas of the same genotype (*Musa*, AAA) known as *Gros Michel* in Uganda and *Williams* in South Africa were specifically studied as representatives of bananas grown in tropical (warmer) and subtropical (cooler) climates respectively and the results are presented in Tables 3.4 (b), 3.5 and 3.6. Spider chart representations of sensory profiles for juices from the three banana cultivars are presented in Figure 3.2 (a), (b), (c) and (d).

In Table 3.4 (a), it is shown that the highest soluble solids (TSS) were 27.1^0Brix found in Kayinja juice where the pulp was treated with Rapidase X-press enzyme. The lowest TSS (15.2^0Brix) was found in the control juice sample extracted from Bogoya. Generally, TSS was higher in juices extracted from the enzyme-treated pulps than in juices extracted from the control pulps in all three banana cultivars.

Titratable acidity (TA) in juices from the three banana cultivars was also higher in the enzyme treated juice samples than in the controls. TA in the juices obtained from the three banana cultivars ranged between 4.5 and 5.8 g/L as anhydrous malic acid. The pH in banana juices ranged from 4.03 to 4.41. The pH, specific gravity, viscosity and turbidity were found higher in the control samples than in the enzymes treated juice samples in all the three cultivars.

The high total soluble solid content of enzyme-extracted juice may be explained by the degradation of the cell wall pectin, cellulose and hemicellulose, resulting in the release of some neutral sugars and leading to a Brix increase. Dorreich (1993) reported that such Brix increase can be as high as 10%. Lanzarini and Pifferi (1989) reported that the use of pectinase can considerably increase the soluble solids and reduce the viscosity of the fruit pulp.

Table 3.4(a) Physicochemical characteristics of juices obtained from three banana cultivars.

Cultivar/Parameter		Enzymes used in juice extraction			
Bogoya (AAA)	**Control**	**Rapidase TF**	**Rapidase CB**	**Rapidase X-press**	**OE-Lallzyme**
TSS (^{0}Brix)	15.2	17.1	17.1	17.4	17.0
TA (g/L)	4.5	5.4	5.5	5.8	5.4
Specific gravity at 20^{0}C	1.13	1.10	1.11	1.11	1.10
pH	4.41	4.04	4.02	4.04	4.03
Viscosity (cP)	12.73	9.91	10.66	10.16	11.03
Turbidity (NTU)	597	531	536	532	537
Mbidde (AAA EA)					
TSS (^{0}Brix)	15.6	18.1	18.7	19.2	18.4
TA (g/L)	4.8	5.7	5.9	6.1	5.7
Specific gravity at 20^{0}C	1.12	1.11	1.10	1.10	1.11
pH	4.32	4.11	4.08	4.12	4.05
Viscosity (cP)	11.06	7.04	7.12	7.07	7.11
Turbidity (NTU)	564	495	493	490	492
Kayinja (ABB)					
TSS (^{0}Brix)	24.5	26.9	26.7	27.1	26.4
TA (g/L)	4.3	5.2	5.1	5.4	5.2
Specific gravity at 20^{0}C	1.14	1.12	1.12	1.11	1.12
pH	4.40	4.08	4.02	4.14	4.13
Viscosity (cP)	16.07	13.02	12.98	13.12	13.06
Turbidity (NTU)	683	612	614	608	614

Data are mans of three experiments replicated.

Sandhu *et al*. (1989) showed that the treatment of Maharaji and Red Delicious varieties of apples with pectic enzyme resulted in an increase in TSS in the juice. Kyamuhangire *et al*. (2002) found the average TSS in banana juice by the enzymatic method (34.9^{0}Brix) significantly higher ($p<0.05$) than in the mechanically extracted juice (30.7^{0}Brix). Our results agree with those previously reported on TSS when pectinolytic enzymes were added to fruit pulps.

Boulton *et al*. (1996) reported that typical ranges for titratable acidity in grape juices are 7 to 9 g/L as tartaric acid and pH was ranging from 3.1 to 3.4. The principal acid (TA) in bananas was investigated (Sadler and Murphy, 1998; Kyamuhangire *et al*., 2002) and found to be malic acid. The pH in this study comparatively showed higher levels in banana juices than in grape juices. The results obtained in this study on TSS, TA, pH and viscosities agree with those of previous researchers (Kyamuhangire *et al*., 2002) who had observed similar trends when they treated the banana pulp with (Pectinex Ultra SP-L), a pectinolytic enzyme.

It had been earlier reported (Rexová-Benková and Marcovic, 1976; Laing and Pretorius, 1992; Gainvors *et al*., 1994, Gonzalez-Candelas *et al*., 1995, Pretorius, 1997) that pectinases de-esterify (pectinesterases) or depolymerise (polygaracturonases, polymethylgalacturonases, pectin and pectate lyases) specific pectic substances. In addition, during the enzyme breakdown of pectin and hemicellulose, unesterified galacturonic acid units are released (Doreich, 1993, Poll, 1993). This may be the reason why there is higher titratable acidity and lower pH in enzyme-treated juices than in the control juice samples. This phenomenon explains the low viscosity and turbidity levels found in the juices obtained from the enzyme-treated banana pulps. The above parameters lowered by addition of commercial enzymes to the pulps would be expected according to pectinolytic activities of enzymes applied.

Table 3.4(b): Physicochemical properties of juices obtained from subtropical *Williams* and tropical *Gros Michel* types of banana (*Musa*, AAA genotype*).*

Parameter	Williams juice	Gros Michel juice
TSS (°Brix)	15.8[a]	19.5[b]
Titratable acid (g/L)	5.4[c]	5.5[c]
Specific gravity at 20°C	1.11[d]	1.12[d]
pH	4.42[cb]	4.46[cb]

Data are means of three experiments replicated.
[a] Different letters in the same row indicate significant difference at $p < 0.05$

The total soluble solids in Table 3.4 (b) are significantly ($p<0.05$) higher in *Gros Michel* juice than in *Williams* juice. The average pH, specific gravity and titratable acidity do not show a significant difference ($p>0.05$) in the values obtained from the two types of *Musa* AAA, genotype.

The high soluble solids (19.5^0Brix) found in the *Gros Michel* juice may be attributed to the longer sunny season in the tropical region being responsible for more total soluble solids (which also implies high sugar content) formation than in subtropical region. The *Williams* juice with 15.8^0Brix grows in the sub-tropical region where there occurs a relatively shorter sunny (cooler climate) season than in tropical region (warmer climate) and thus ends up with less TSS formation and distribution in the fruits. The results of TSS observed in *Gros Michel* and *Williams* in this study confirm what IITA (1993) had reported that chemical composition of bananas varies, and the variations are reported to be the result of many factors, including ecological location,

nutrition, location on the bunch from which the banana fingers are sampled for analysis, and maturity of the fruit at harvest.

Regarding the relationship between enzymes and pH, every enzyme requires a specific pH for its optimal activity. This pH is of paramount importance when choosing an enzyme for industrial process. For example, for clarification of an acidic fruit juice pH<3, an enzyme exhibiting a pH range of 4-5 would show less activity at pH 3. For maximum enzyme efficiency, enzymes with optimum activity at a specific pH have to be strategically chosen (Uhlig, 1998).

3.3.3 Effect of enzymes on turbidity of the banana juices

The results of turbidity reduction in juices extracted after treatment of banana pulp with the pectinolytic enzymes and their controls are presented in Table 3.4 (a) and Table 3.5. The best turbidity reduction was obtained with the banana pulp treated with Rapidase X-press, measuring a turbidity of 490 NTU in the Mbidde juice. In the juices extracted from pulps treated with enzymes (Table 3.5), there was no significant difference ($p>0.05$) in turbidity levels of *Williams* or *Gros Michel* juices but there was a difference ($p<0.05$) between the turbidity levels of juices from the two types of *Musa*, AAA genotype. The control juices also showed a very significant difference from the juices obtained from enzyme treated banana pulps.

The freshly extracted juices generally had turbidity ranging from 490 to 614 NTU in the juices obtained from enzyme-treated pulps while in the control juices, the range was between 564 and 683 (Table 3.4 a) in all 3 cultivars.

The value of pectinases and their effectiveness at fruit pH and temperatures of up to 55^{o}C was demonstrated by Koch (1956) not only for clarification of fruit juices but also for an improved pressing of chopped fruits, called fruit mash. Uhlig (1998) further said that current enzyme preparations used in fruit juice and wine making posses specific capabilities for degrading hydrocolloids, depending on the raw material and the product desired. The enzymes facilitate the sedimentation of colloidal particles resulting from degraded banana pectins, galactans and arabino-galactans (Colagrade *et al.*, 1994; Haight and Gump, 1994). It was stated by Dorreich (1993) and Sole (1996) that commercial and experimental banana juice production must be based on the enzymatic method which allows ready separation of clear juice. The juices obtained from enzyme-treated banana pulps in this study were generally less turbid than those from untreated pulps. The results obtained are in agreement with findings of the above researchers who used different commercial enzymes on various fruits in their juice clarity investigations. Pectinolytic enzymes played a significant role in turbidity reduction in the banana juices.

Table 3.5: Effect of pectinolytic enzymes on the turbidity of banana (*Musa*, AAA genotype) juices**.**

Enzyme	Williams juice(NTU)	Gros Michel juice(NTU)
Rapidase X-press	532±0.57^{a}	545±0.28^{b}
Rapidase-CB	536±1.7^{a}	547±0.78^{b}
Rapidase-TF	531±1.20^{a}	543±0.53^{b}
OE-Lallzyme	537±1.69^{a}	549±0.001^{b}
Control	597±0.28^{c}	619±0.212^{d}

Values are means ± standard deviation of three experiments replicated.
^{ab}Different letters in the same row indicate significant difference at $p<0.05$.
^{b} The same letter in the same column indicates no significant difference at $p>0.05$.

3.3.4 Effects of enzymes on viscosity of the banana juices

The results of viscosity reduction effected by the enzymes used in this study are presented in Table 3.4 (a) and Table 3.6. The juice extracted from Mbidde pulp treated with enzyme Rapidase X-press had the lowest juice viscosity of 7.07. The viscosity of the juices remained highest in the control juices for the 3 cultivars. There was no significant difference ($p>0.05$) in juice viscosity reduction between the banana subgroups of *Williams* and *Gros Michel* treated with the same enzyme. That implied that the enzymes had the same effect on both of the *Musa*, AAA genotype types. The viscosity range was found between 7.07 and 13.12 cP and between 11.06 and 16.07 cP in the enzyme treated and untreated pulps respectively. The four commercial

enzymes used showed a significant difference ($p<0.05$) in juice viscosity reduction compared to their respected controls. This difference may be attributed to the fact that different pectinolytic enzymes have different capacities to degrade and rapture juice cell structures by breaking the banana cellulose, hemicellulose and pectin. The role of pectolytic enzymes discussed in sections 2.7.1 and 2.7.4 shades more light on viscosity reduction in fruit processing. Previous research results on bananas have reported that ripe bananas contain approximately 3-4% total fibre (Paul and Southgate, 1978), with about 1% cellulose (Southgate, 1969), 0.5-1% pectin (Kawabata and Sawayama, 1974), and 1-2% hemicellulose (Berell, 1943). Ripe banana also may contain 1-4% starch (United Fruit Co., 1961) depending on how uniformly ripe the fruits being processed are. All the above mentioned complex substances in the banana fruit have the capacity to make juice extraction a cumbersome process. Therefore, it is of paramount importance to apply appropriate enzymes in the pulp that can degrade complex polysaccharides to enable juice release and filterability. Mabesa *et al*. (1989) noted that banana juice was more easily pressed from the enzyme treated pulp as compared to the untreated pulp. And they suggested that, this could be the result of the decrease in the viscocity of the juice due to the solubilisation of the pectin. Koffi *et al*. (1991) showed that mixtures of pectinase, cellulase and hemicellulase, when used at recommended rates, were more effective than pectinase, cellulase, alpha-amylase or galactomannanase on their own in reducing viscosity and improving the filterability of puree from both green and ripe bananas. When we compare previous results presented in Table 2.8 by Kyamuhangire *et al.* (2002) on pure juice yield from Kayinja (*Musa*, ABB genotype) and those obtained in this study, it can be seen that juice yields influenced by enzymatic action on Kayinja banana mash was different when different pectinolytic enzymes were used, though conditions (temperature, enzyme and enzyme contact time) between our experiments and Kyamuhangire *et al.* (2002) were not the same. Nevertheless, the juice yields influenced by the addition of enzymes to banana pulps were higher than that extracted mechanically and to the control. Future investigations may necessitate finding out the most appropriate enzyme mixtures for viscosity reduction, temperature and enzyme-mash contact time for maximum juice yields in various banana cultivars.

Table 3.6: Effect of the pectinolytic enzymes on the viscosity of Bogoya *Musa,* genotype AAA juice measured at 20^0C.

Enzyme	*Williams* Juice (cP)	*Gros Michel* Juice (cP)
Rapidase X-press	10.16±0.014[b]	10.29±0.007[b]
Rapidase-CB	10.66±0.007[bc]	10.58±0.014[bc]
Rapidase-TF	9.91±0.014[a]	9.97±0.014[a]
OE-Lallzyme	11.03±0.07[d]	11.12±0.007[d]
Control	12.73±0.007[e]	12.89±0.007[e]

Values are means ± SD of three experiments replicated.
[a] Different letters in the same column indicate the significant difference at $p<0.05$.
[b] The same letter in the same row indicates no significant difference at $p>0.05$.

3.3.5 Sensory characteristics of banana juices

The results of sensory tests for different attributes are presented in Figures 3.2 (a), (b) , (c) and (d) and represent mean scores of the sensory characteristics on the nine-point hedonic scale. The juice attributes focused on for assessment were flavour, taste, aroma, acidity, mouth feel and overall acceptance. Flavour (a combination of taste and aroma) was related to banana flavour. The most typical banana flavour as observed by the panel was for Kayinja juice and as a result Kayinja juice scored highest in overall acceptance, with scores between 7 and 8 points on the nine-point scale. Acidity which was assessed based on sourness of juice was highest for Bogoya juice and lowest for Kayinja juice. The colour intensity of the juices from the three cultivars scored between 6 and 7.5, whereas mouth feel assessed in terms of smoothness scored between 5.5 and 7 points. Bogoya juice extracted with the aid of the enzyme Rapidase X-press, which yielded the highest juice volume, was rated the lowest in overall acceptance. Rapidase X-press treated juices scored the lowest on the nine-point hedonic scale for all the attributes tested. This may imply that whereas Rapidase X-press is capable of producing higher juice yields compared to the other enzymes, it seems somehow to affect the sensory quality of the product. The control samples of all three cultivars scored the highest in overall acceptance. This implies that the enzymes used to treat banana pulps may have slightly altered some of the attributes negatively. Overall acceptance of the juices obtained from the control pulps in the three cultivars scored 7.1 (Figure 3.2 d) on the nine-point hedonic scale and this was the highest average score. The lowest (6/9) acceptance score was for juices extracted from pulp treated with Rapidase X-press enzyme. Kyamuhangire *et al*. (2002) reported that the overall acceptability of enzyme-treated juices not scoring very high may be a result of a slight astringent

taste as observed in their study where juices extracted by mechanical and enzymatic methods was compared. Although we did not measure astringency as one of our attributes it is tempting to speculate that the lower overall acceptability may be due to the same reason. The control samples of all three cultivars scored the highest in overall acceptance. This implies that the enzymes used to treat banana pulps may have slightly altered some of the attributes negatively.

The possibility of the enzymes used in juice extraction to slightly affect the attributes against preferences of tasters is related to what Vilanova *et al.* (2000) had reported (though their work was on wine not juice). They reported that with reference to wines obtained from must supplemented with commercial pectolytic enzymes, in as far as typicality was concerned, the wines display aromas that are less typical or not typical at all due to the release of terpenes and esters, a consequence of the action of some of the commercial enzymes. The results of our study seem to agree with the previous observations especially when Rapidase X-press enzyme was used. While Rapidase X-press produces the highest juice yields, it may not be the best choice for processing a ready to drink (RTD) beverage from bananas when consumer acceptance is desired. Perhaps, mixing different commercial pectinolytic enzymes or blending different batches of banana juices may improve the organoleptic qualities and general acceptance. Kyamuhangire (1990) noted that the high (29^0Brix) total soluble solids content of banana juice was an indication of high sugar content and reported that banana juice extracted from Lady-finger bananas had 25^0Brix with 21.1% total sugar. Earlier on, Wills *et al*. (1986) reported that bananas may contain up to 17.3-18.2% sugar. This may explain why the Kayinja juice was the most preferred juices because of the higher sugar content.

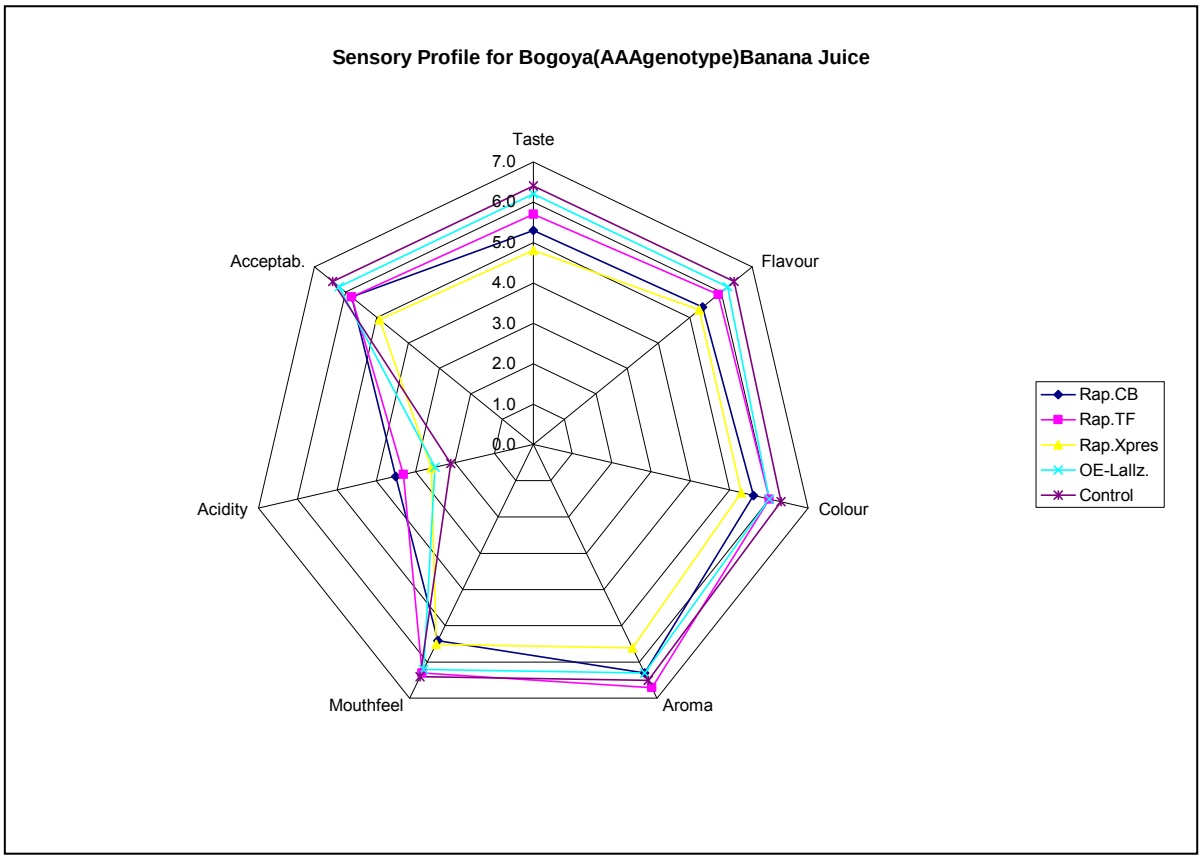

Figure 3.2 (a): Spider chart representation of sensory profile for Bogoya (*Musa* AAA genotype) banana juices.

In Bogoya juice, Figure 3.2 (a), aroma was assessed highest in juice treated with Rapidase TF. The control had the highest mouthfeel. Aroma was scored lowest in Rapidase X-press treated juice whereas mouthfeel was assessed lowest in both juices treated with Rapidase X-press and Rapidase CB respectively. Colour, flavour, taste and overall acceptability were assessed highest in the control juice. On the other hand, colour, flavour, taste, aroma and acceptability were lowest in Rapidase X-press treated juices. Acidity was assessed highest and lowest in Rapidase CB and OE-Lallzyme treated juices respectively. These effects on Bogoya juices may be related to the different enzyme activities earlier described in the commercial enzyme preparations in Table 3.1.

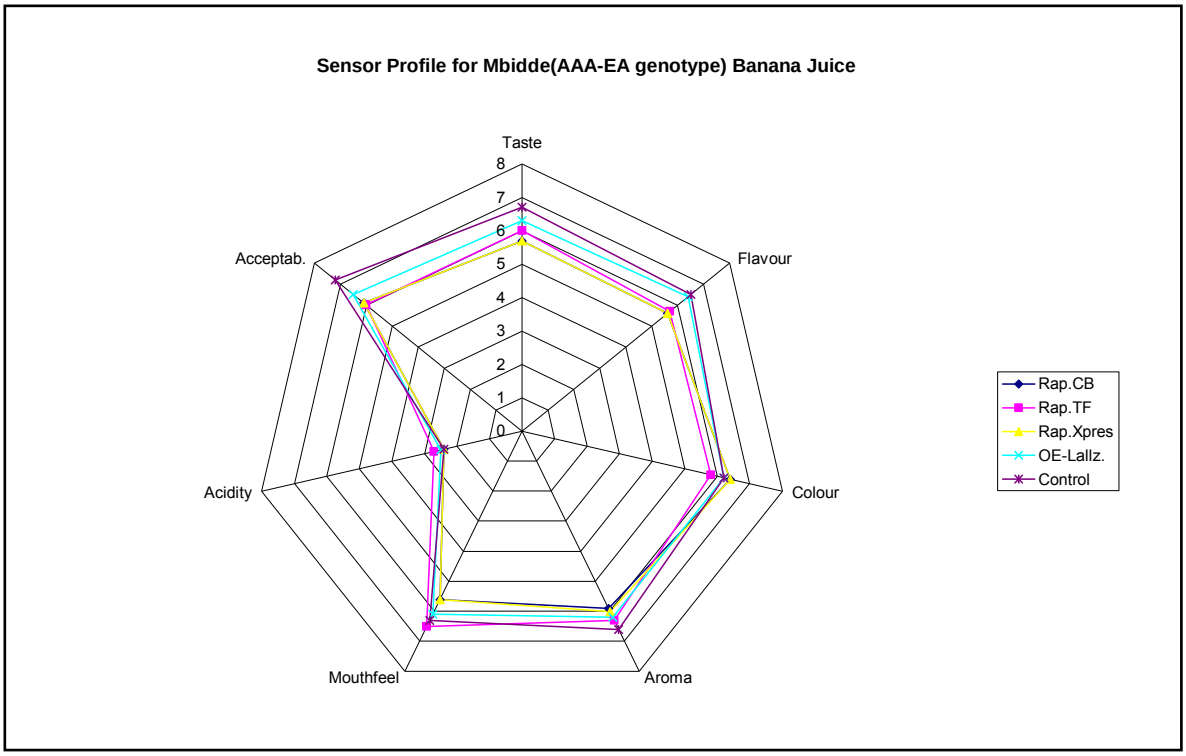

Figure 3.2(b): Spider chart representation of sensory profile for Mbidde (*Musa* genotype AAA-EA) banana juices.

In Mbidde juice, Figure 3.2 (b), aroma, flavour, taste and acceptability were also assessed highest in the control juice. The same attributes scored least in Rapidase X-press treated juice. Colour scored highest and lowest in the juices treated with Rapidase X-press and Rapidase TF respectively. Mouthfeel scored highest in the Rapidase TF treated juice and lowest in the Rapidase X-press treated juice. Acidity was highest and lowest in Rapidase TF treated and control juices respectively. Once again these effects on Mbidde juices may be related to the different roles played by the different enzyme activities earlier described in the commercial enzyme preparations.

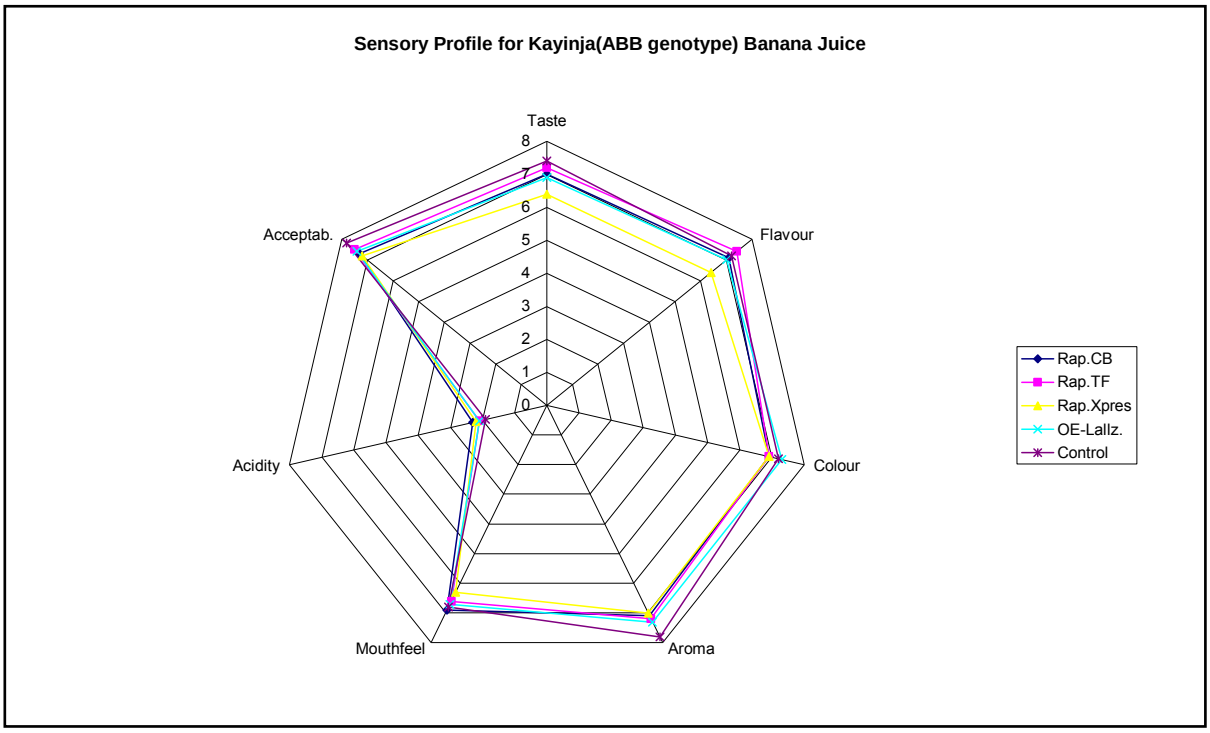

Figure 3.2 (c): Spider chart representation of sensory profile for Kayinja (*Musa*, genotype ABB) banana juices.

In Kayinja juice, Figure 3.2 (c), mouthfeel, aroma, taste and acceptability scored most in the control juice. The same attributes scored least in Rapidase X-press treated juice. Flavour scored most in Rapidase TF treated juice and least in Rapidase X-press treated juice. Colour scored most in OE-Lallzyme treated juice and least in both Rapidase TF and Rapidase X-press treated juices. Acidity was highest and lowest in Rapidase CB treated and control juices respectively

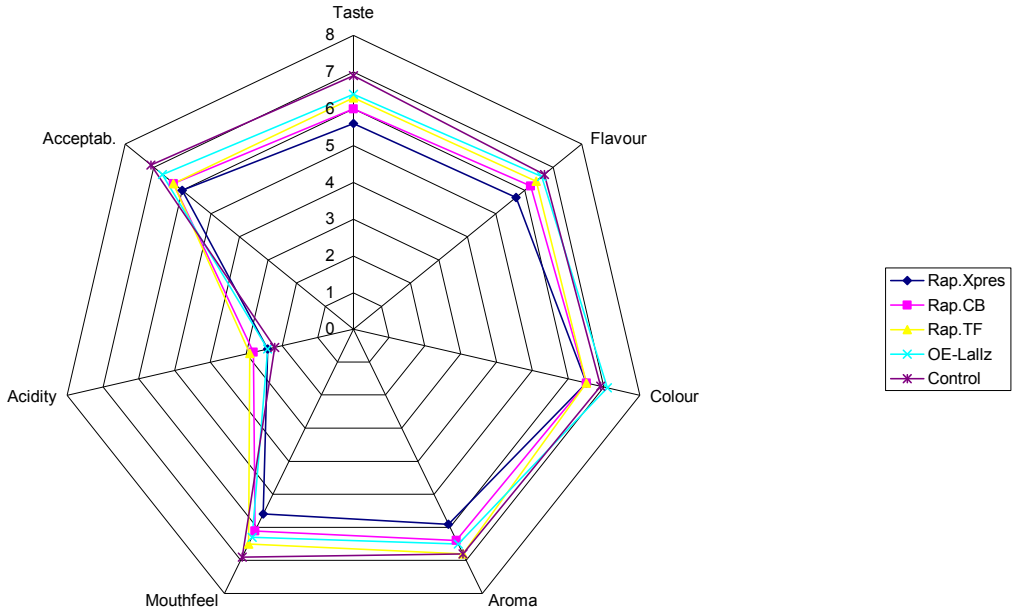

Figure 3.2 (d): Spider chart of the mean values of the attributes assessed in the juices obtained from all three cultivars for each enzyme used.

The mean values of each attributes tested for all three banana cultivars together for each enzyme used is presented in Figure 3.2 (d), the sensory profile showed most of the attributes scoring highest and lowest in juices obtained from the untreated juices (controls) and Rapidase X-press treated sample respectively.

The lowest (5.6/9) and highest (6.9/9) acceptability scores was obtained for juices from Rapidase X-press treated and control pulps respectively. Flavour also scored lowest (5.7/9) and highest (6.7/9) in juices obtained from Rapidase X-press and control pulps respectively. Colour scored lowest in juices obtained from Rapidase X-press, Rapidase CB and Rapidase TF pulps at the same level (6.5/9) on the nine-point hedonic scale and highest (7.1/9) in juice obtained from OE-Lallzyme treated pulp. The aroma scored lowest (5.9/9) in Rapidase X-press treated pulps and highest (6.8/9) in juices obtained from Rapidase TF treated pulps and the control. Mouthfeel scored lowest (5.6/9) in juices obtained from Rapidase treated pulps and highest (6.9/9) in juices obtained from the control pulps. The acidity was assessed lowest (2.2/9) in juices obtained from the control pulps and highest (2.9/9) in juices extracted with Rapidase TF.

The overall acceptability of juices from three banana cultivars scored lowest (6.0/9) in the juices obtained from Rapidase X-press treated pulps and highest (7.1/9) in juices obtained from control(non-enzyme treated) banana pulps.

Results of this sensory evaluation showed Rapidase X-press scoring lowest in attributes (especially taste, flavour, aroma and acceptability) that would promote the acceptance of banana juice by consumers. Considering the effects of Rapidase X-press on sensory attributes affected as discussed above, it may not be advisable to encourage use of this particular pectinolytic enzyme for juice extraction if the quality of juice is to be based on the attributes tested.

Vilanova *et al.* (2000) had reported that with reference to wines obtained from must supplemented with commercial pectolytic enzymes, as far as typicality is concerned, these wines display aromas that are less typical or not typical at all due to the release of terpenes and esters, a consequence of the action of some of the commercial enzymes. Although the slight changes were noted by the panel in the juices in this study, it may be because of similar effects that as reported by the above researchers.

It is important to realise that the differences in the attributes assessed under different enzyme treated juices in the same banana cultivar was not statistically different.

However, there was a still a slight difference in the sensory profiles of the three banana cultivars that were studied. The banana juice from Kayinja was most preferred by panellists.

The results of this study showed that the TSS in the juices extracted from the 3 banana cultivars ranged from 15.2 to 27.1^{0}Brix. The TA ranged between 4.3 and 6.1g/L. Both TSS and TA were higher in juices obtained from the enzyme treated pulps. The pH ranged from 4.02 to 4.41 while the viscosity and turbidity ranged from 7.04 to 16.07cP and from 490 to 683 NTU respectively. The pH, viscosity and turbidity were lower in the in the juices obtained from the enzyme treated pulps compared to the control juices. The increased TSS and decreased viscosity and turbidity are of significant importance for banana beverage processing because the increase in sugar in the juice seems to make it more acceptable by the consumer and the decreased viscosity and turbidity improve processing of the product.

Therefore, the enzymatic processing used in this study seems to be a basis for future banana juice production development, following the results so far achieved. The overall acceptability could be improved on either by manipulations during juice processing such as mixing enzymes and blending different juices batches. Based on our research findings, pectinolytic enzymes may prove very useful in application of research and development (R & D) programs in the industrialisation of banana juice.

3.4 CONCLUSIONS

We can conclude that an acceptable quality banana juices can be produced from all three banana cultivar, even if the bananas are over-ripe. Results of this study showed that the commercial pectinolytic enzymes used played a significant role in increasing banana juice yields, total soluble solids and improving juice clarification. The highest juice yield was obtained from Mbidde cultivar, which is traditionally known as a "juice type" of banana because of its capacity to release free-run juice. G*ros Michel* and W*illiams* proved to be reliable sources of juice, and may therefore be utilised equally well for banana juice production in the overripe form as an alternative to its traditional use as a dessert banana. The TSS of the bananas that belong to the same genotype (M*usa* AAA) was significantly different. Turbidity was also significantly reduced when enzymes were used.

These results form the bases for processors trying to produce high quality clarified juice from over-ripe bananas that normally are not consumed in the over-ripe state.

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CHAPTER 4

RESEARCH RESULTS: THE INFLUENCE OF COMMERCIAL ENZYMES ON WINE CLARIFICATION AND ON THE SENSORY CHARACTERISTICS OF WINES MADE FROM THREE BANANA CULTIVARS

Abstract

The purpose of this study was to apply enzyme preparations to banana pulp to improve wine clarity, alcohol yields and to assess sensory properties. The ultimate goal is to produce quality and safe banana wine with a stable shelf live. Commercial enzymes were applied to banana (*Musa spp*.) pulp. The materials used in the study included four selected commercial enzyme preparations and banana juices extracted from pulps of three banana cultivars. Compared with the control, the wine turbidity was lowered significantly in the wines prepared from juices that were extracted from commercial enzyme treated pulps. The effect of proteases on protein hazes was also investigated after periods of one week and four weeks. The addition of proteases had a significant effect on the protein hazes ($p<0.05$). A longer incubation period resulted in greater reduction in turbidity. The alcohol yields were significantly better ($p<0.05$) in the wines prepared from juices extracted from pectinolytic enzyme treated pulps than in the untreated pulps. The general organoleptic quality of the wine did not show significant difference ($p>0.05$) from the control according to the ranking attributes assessed by the panellists. The categories that were consensually derived by the panel of tasters in the flavour profile of the banana wines prepared from three cultivars included wine sweetness, acidity, sourness, fruitiness, astringency, aromatic and dryness. Overall acceptance of the banana wines by the panel scored five and a half points (5.5/9) on the nine-point hedonic scale.

Keywords: Pectinolytic enzymes, banana cultivars, proteases, banana wine

4.1 INTRODUCTION

Tropical fruit juices are among the most consumed beverages on market today. These juices can also be processed into wines although the term wine is usually associated with beverage made from grapes. The premium raw material for winemaking is the grape. The composition of wine is complex and the concentration of the components occurs in varying amounts as presented in the Appendix 5(a) and 5(b). This composition of wine gives it a unique shelf life which can mature into a superior product as long as it is hygienically processed and aged in aseptic conditions.

Wine is one of the oldest alcoholic beverages dating back to the Egyptians, almost 5000 years ago (Bartowsky and Henschke, 2004; Pretorius, 2000). Until the early years of the seventeenth century, wine was considered to be the only wholesome readily storable beverage, accounting for the rapid global increase of wine fermentation technology (Pretorius, 2000). Wine plays a major role in the economies of many nations, which produce more than 26 billion litres of wine annually. Modern winemakers supply a variety of wines year round independent of location and time of consumption. Fierce competition has led to increased diversity and innovation within the wine industry, much to the benefit of the consumer (Pretorius, 2000).

It is generally accepted that moderate wine drinking is socially beneficial and can be effective in the management of stress and reduction of coronary heart disease. The principal protective compounds found in wine include the phenolic compounds, resveratrol, salisylic acid and alcohol (for a detailed review on improvement of wholesomeness of wine, see Pretorius and Bauer, 2002).

The yield of alcohol is of obvious practical importance to the wine maker (Amerine *et al*., 1980). According to Gay-Lussac equation, the theoretical yields of alcohol are 51.1% and carbon dioxide is 48.9% by weight of glucose fermented. This is biologically unobtainable and in practice will depend on a variety of factors, *viz*: amount of by-products, amount of sugar used by yeasts, sugars used by other microorganisms, alcohol lost by evaporation or entrainment (which in turn partially depends on temperature and the rate of fermentation), presence of air, stirring or other movement fermenting mass, and other factors (Amerine *et al*., 1980).

Different fruit juices have been used in wine making though grape wine remains the superior wine. Bananas have also been used as a substrate for winemaking, but due

to problems of poor post-harvest handling (Joshi *et al.*, 2000; Akingbala *et al*., 1994), processing, and packaging and consequently a lack of value-added products, banana beverage products are still limited on both regional and international markets. Local methods of processing banana beverages are commonly used, but, in most cases, these methods yield inferior banana beverages. A traditional technique based on the physical working out of pulp mixed with spear grass (*Imperata cylindrica*) until the juice is released has been employed for years (Kyamuhangire *et al*., 2002). While the sensory properties (taste and aroma) may have been achieved with this traditional approach in the local communities, extraction and clarification efficiency have remained low for the production of quality banana beverages for a competitive market with a variety of fruit beverages. The main problems encountered in banana beverage processing have been mainly due to difficulty in juice extraction and turbidity of the beverages. However, the application of enzymes, particularly for extraction and clarification, has achieved better clarification and improved the general yields. Such achievements in different banana cultivars have been reported (Viquez *et al.*, 1981; Kyamuhangire *et al.,* 2002). Traditional banana juice extraction and its subsequent fermentation to produce banana beer is an important social and economic activity among many tribes in East Africa (Munyanganizi-Bikoro, 1975; Stover and Simmonds, 1987; Davies, 1993). Although there is a lot of banana wastage due to fruit injuries resulting from poor handling and transportation methods (Viquez *et al*., 1981), improved methods of processing can tremendously reduce fruit wastage, since bananas rejected on fresh-fruit markets due to external injury are a potential source of raw materials for juice processing (Viquez *et al*., 1981; Shahadan and Abdullah, 1995). Such banana juice is suitable for banana winemaking. Banana wine processing at laboratory and small-scale production levels without the use of commercial enzymes has been successful to some extent (Akingbala *et al*., 1994; Joshi *et al*., 2000; Lewis, 2002).

In this study, we report on the influence of commercial enzymes on banana wine clarification and on the sensory characteristics of wine made from the three banana cultivars, *viz*. *Musa* ABB (Kayinja), AAA (Bogoya) and AAA-EA (Mbidde) genotypes.

4.2 MATERIALS AND METHODS

General grape wine production methods were adapted for banana wine production, with an aim of obtaining a stable and acceptable safe banana wine product.

4.2.1 Banana cultivars used and pulp preparation

The banana cultivars used and the pulp preparation for extraction of juice for banana winemaking was that shown in section 3.2.1 in chapter three. Part of the juice was reserved for further studies about juice characteristics and the other juice was used for banana wine preparation.

4.2.2 Enzymes used

The enzymes used were described in section 3.2.2 of chapter three. However, additional enzymes (proteases) were used to study haze stabilization in wine. Two protease enzymes viz: Zumizyme and Papaine were applied to stabilise protein hazes in wine. Zumizyme AP was obtained from Bio Bright in Japan and Papaine was got from Novozymes SA. These proteases were applied at a dosage of 0.2 g/L to wine as recommended by the suppliers. The enzymes that were used for studies on banana wines are presented in Table 4.1.

Table 4.1: The enzymes used for banana wine processing

Enzyme	Description	Dose	Producer
Rapidase X-press	Pectinase activity	0.03 g/kg	Gist-brocades
Rapidase CB	Pectinases, Maltodextrine, NaCl	0.03 g/kg	Gist-brocades
Rapidase TF	Pectinase, hemicellulase, cellulase activity	0.03 g/kg	Gist-brocades
OE-Lallzyme	Pectinase activity	0.03 g/kg	Lallemand
Zumizyme AP	Protease	0.2 g/L	BioBright
Papaine	Protease	0.2 g/L	Novozymes SA

4.2.3 Juice extraction

Juice extraction was done as described in section 3.2.3 of chapter three. Part of the juices extracted was used for studies on juice characteristics and the rest of the juices were used for banana winemaking.

4.2.4 Banana winemaking

Juices were extracted after 24 hour reaction time from the pulps treated with the four selected enzyme preparations, viz. Rapidase X-press, Rapidase CB, Rapidase TF, and OE-Lallzyme. The controls were reserved as untreated (no enzyme added) samples for each banana cultivar. The total soluble solids, pH and titratable acidity of the juices were determined. Oxidation and polyphenoloxidase (PPO) browning effects were prevented by the application of 50 ppm of sulphur dioxide in the juice. Diammonium phosphate (DAP; Lab Scientific Equipment, Cape Town, South Africa) was added to adjust the nitrogen concentration of the banana juice depending on the free available nitrogen (FAN) concentration of the pulp. The amount of DAP added was calculated using the formula below for estimating the assimilable nitrogen required as established by Vos *et al*. (1980):

$$\frac{43.9\text{-FAN}/\,^{o}\text{B}}{0.108} \times 0.5 = \text{g/hL DAP}$$

The extracted juices were inoculated with a *Saccharomyces cerevisiae* yeast strain (VIN 13) at a concentration of 0.3 g/L. The yeast was weighed and dissolved in 20 mL of banana juice and incubated at 37^{0}C for 20 minutes. Fermentation was conducted in 2.5 L bottles at a desired temperature of 15°C. The fermentation progress was monitored by measuring the decrease in weight of the fermentation bottles, and alcoholic fermentation was considered complete when the weight of the bottles remained constant for three consecutive days. Upon completion of alcoholic fermentation, the wines were cold stabilised at a temperature of -4°C for four days. Wines were racked off the lees after settling. The wines were then filtered through sterile semi-permeable 300 µm filter pads at a pressure applied between 50 and 150 kPa. The filtered (limpid) banana wines were sampled for physicochemical and sensory analyses. Other wines samples were bottled and stored for flavour profiling on later stage.

4.2.5 Physicochemical analyses of the banana wine

A number of physicochemical properties were analysed. Total soluble solids (TSS) were determined in the wine by using a hydrometer (^{0}Balling) as described by Iland

et al. (2000). Titratable acidity and pH were automatically determined with the use of a 20-702 SM Titrino (Metrohm) and with a glass calomel electrode pH meter (Cole Parmer, Vernon Hills, IL, USA) respectively. Samples of wine (50 ml) were titrated with 0.1M sodium hydroxide solution with an indicator for the determination of TA. Reducing sugars were analysed by the Rebelein method, described by Iland *et al*. (2000). Volatile acid (VA) was measured using a B & M Scientific Markham Still. Turbidity was measured by using a digital Hack turbidimeter and expressed in nephelometric turbidity units (NTU). The alcohol content of the wine was determined using an alcoholmeter (CDS-Germany), immersed in a distillate at a temperature of 20^0C and expressed in percentage volume by volume (% v/v).

4.2.6 Distillations

Some of the banana (*Musa*, ABB genotype) wines were also singly distilled in electrically heated round-bottomed 1 litre flasks. Antifoaming agent was added to prevent foaming on heating wines. Boiling chips were added to ensure homogeneous heat distribution during distillation process. Temperature was controlled and kept around 78± 5^0C, the boiling point of ethanol. A Liebig condenser system was used to collect the distillate heads. Distillation proceeded until the alcohol-water mixture in the flask was approximately 50% by volume evaporated. The distillate samples were collected and kept tightly sealed in 50 ml bottles at ambient temperature prior to gas chromatography (GC) analysis**.**

4.2.7 Gas chromatography

The following apparatus and chromatographic conditions were used for the detections: An HP 6890 series gas chromatograph fitted with a flame ionization detector (FID), a split-splitless injector, and automatic sampler 7683. A Supelco SPB5 column: (60 m x 0.32 mm i.d., 0.25 μm film thickness) was used. Chromatographic conditions entailed the following: He as carrier gas, head pressure of 140 kPa; total flow of 12.5 mL/min; purge flow of 7.0 mL/min; injector and detector temperature of 250°C; initial column temperature of 50°C, held for 2 min and then raised to 150°C at 10°C/min, then to 160°C at 5°C/min and then to 220°C at 10°C/min and held for 10 min; make-up gas N_2 at 30 ml/min; detector FID, H_2 at 40 mL/min; air, 450 mL/min; injected volume, 2 μL.

The following method was used: 10 mL of wine was introduced into the extraction tube, and 200 μL of Freon 113 (1, 1, 2-Trichloro-1,2,2-trifluoroethane, obtained from Sigma-Aldrich) was added as extracting agent, as well as 2 μL of a solution of 2,6-dimethylheptenol (400 mg/L in ethanol as internal standard). NaCl (1.2 g) was also added. The tubes were capped and shaken for 30 min in automatic shaker at maximum speed. The tubes were centrifuged for 5 minutes at 3000 rpm (with a g-force of 2200) and the organic phase was recovered with a Pasteur pipette, transferred over 50 mg Na_2SO_4 into a HP 2 mL vial with a 200 µL glass insert, and analyzed under the chromatographic conditions described above. After the chromatographic analysis, the relative areas or heights of the calibrated peaks were interpolated from calibration graphs created with synthetic wine solutions (ethanol 12% for white wine, 16% for red wine v/v; tartaric acid 6 g/l; pH 3.2) having an alcohol content similar to that of the analyzed wine.

4.2.8 Sensory evaluation of banana wines

Ranking sensory attributes

Before the formal evaluation started, the panellists were subjected to a two-hour training session to become familiar with the sensory attributes of the banana wine to be assessed. Each judge was introduced to a sensory evaluation score card and briefed on the procedures of evaluation during the training. A ready-to-drink (RTD) banana beer was presented as reference. To check the assessor's consistency, a replicate of the wine was presented. A nine-point rating system was used.

Sensory evaluation was conducted by 20 members of academic staff and students at the Food Processing Laboratory, Department of Food Processing Technology, Kyambogo University. These could be considered banana beverage connoisseurs because of their general knowledge, interest and experience in local banana beverages. For each test, the panellist was served with 50 ml of banana wine in 100 ml wine glasses. Coded wine samples that had been cooled at 7°C for 24 hours were served. The panellists were instructed to rinse their mouths with water after tasting each of the wine samples provided.

The sensory attributes assessed by the panel of tasters were ranked from low to high levels on a nine–point hedonic scale. Taste was measured in terms of sweetness,

whereas flavour and aroma were related to the banana flavour. The mouthfeel was assessed in terms of smoothness and astringency of the wine on the tongue. Acidity was assessed in terms of sourness of the wine in the mouth. Colour of the wine was evaluated in terms of its intensity in wine. The overall acceptability was the general preference expressed by the assessors after evaluating the attributes in the sensory profile. The score values for the different attributes were subjected to ANOVA test to determine if there were significant differences between the juices.

Descriptive analysis of wine flavour profiles

The initial screening for general ability of assessors was based on primary tastes (ISO 3972, 1991; Jellinek, 1985). The standard solutions used for training were:

- sweet - 4g/L;10g/L and 16g/L sucrose;
- salt - 1g/L;2g/L and 3g/L sodium chloride;
- acid - 0.2g/L;0.5g/L and 1g/L tartaric acid;
- bitter - 0.01g/L;0.015g/L and 0.02g/L quinine sulphate.

Several training sessions attended by twelve members of Uganda Coffee Development Authority (UCDA) were conducted. Several fruits i.e. passion, pear, "*sweet ndiizi*" mango, grapefruit, orange, apple, mandarin, lemon and lime juices were used in training sessions to acquaint panellists with different flavours and relate them to banana wine. The tests were carried out between 09:00 and 11:00 am each day in the laboratory.

Five assessors were selected out of the twelve that had initially started the training sessions. They were further trained through several sessions to generate descriptors freely and confirm them in a consensus agreement.

Banana wine made from pulp treated with enzyme Rapidase X-press was obtained from each cultivar and presented to the panel members to assess if the wines had differences in their flavour profiles. To assess flavour profile of wine from the same cultivar but treated with different enzymes, five wines were obtained from Mbidde, four of them enzyme treated and the fifth sample as the control.

The members of the panel were asked to describe the wine flavour with as many descriptors as they could and then confirm the descriptors through discussion to come up with a consensus wine profile. The panellists tasted one sample at a time, described it and rinsed their mouth with cool boiled water before tasting another sample.

4.2.9 Statistical analysis

Analysis of variance (ANOVA) was performed for all the wine yields and turbidity of the wines (Zar, 1984). ANOVA was also performed for the viscosity of the wines. This was done using the STATISTICA 7 processing package.

4.2.10 Haze stabilisation

The protease enzymes that were applied for haze stabilization were Zumizyme and Papaine. These enzymes were applied to bottled banana wine that had been stored at ambient temperatures for four months and had developed some haze. The rate of application of the enzymes was 0.2 g/L. The initial haze level before treating the wine with protease enzymes was 58.3 nephelometric turbidity units (NTU). An untreated sample was reserved as a control. Three trials were carried out in duplicate for a period of one week and four weeks. The samples were observed in reference to turbidity reduction (limpidity), using an electronic turbidimeter after the set period and units were expressed in nephelometric turbidity units.

4.2.11 Microbiological analysis

Microbiological analysis was conducted on the bottled wines after one week, one month and three months of shelf life storage. The analyses were carried out in the Food Section Laboratory, at “Government Chemist”, Kampala, Uganda. A standard Gram-staining procedure was used to identify bacterial isolates; Gram-negative acetic acid bacteria (AAB) which appear red and Gram-positive lactic acid bacteria (LAB) which appear purple blue under the microscopic.

Bromothymol Blue-Lactose broth was used to investigate if there are any coli-forms present in the young banana wines. Using the 3-tube MPN method (Corry and Curtis, 1999), dilutions of the wine samples were done using 0.1% peptone water as the diluents. Durham tubes were inserted into the fermentation tubes to trap any gas produced. Incubation was done in triplicates at 37°C for 48 hours. Any tubes containing gas would be considered positive. The results were calculated using the MPN (most probable number) table.

Total viable count was done using standard nutrient agar. After decimal serial dilutions, 0.1 ml of the diluted sample was spread out on the medium using sterile Conrad's rod. Microbial growth was expected after incubation at 37^0C for 24 hours.

For any yeast count, decimal serial dilutions were done as indicated above. Portions of 0.1 ml were spread on Rose-Bengal agar and incubated (in triplicate) at 37^0C for 48 hours as described by Jackson and Badrie (2002). Total numbers of aerobic mesophilic viable micro-organisms, acetic acid bacteria, lactic acid bacteria and yeasts were to be enumerated as colony forming units per millilitre (cfu ml^{-1}) on nutrient agar (Oxoid Limited, Hampshire, UK), as described by Fugelsang (1997).

4.3 RESULTS AND DISCUSSION

4.3.1 The effect of pectinase and protease enzymes on turbidity of banana wine

Results showing the different effects on the banana wines caused by using pectinolytic and proteolytic enzymes during the wine preparation and maturation processes are presented in Table 4.2 and Figure 4.2 respectively. All the commercial enzymes showed a significant reduction in turbidity. The OE-Lallzyme was the most effective enzyme in turbidity reduction in all the wines from all three banana cultivars, followed by Rapidase TF in Bogoya (*Musa*, AAA genotype) wine. These were followed by Rapidase CB and Rapidase X-press in Bogoya wine. Rapidase CB, Rapidase TF and Rapidase X-press caused almost the same effect in Mbidde (*Musa*, AAA-EA genotype) and Kayinja (*Musa,* ABB genotype) wines respectively. The most turbid wine among the enzyme treated wines was the Rapidase CB enzyme treated one, with turbidity of 12.67 NTU obtained from the Kayinja banana cultivar. If maximum degradation of polymers is desired, cellulases and hemicellulases are combined with pectinases (Uhlig, 1998). Similar enzyme cocktails were used in this study. The turbidity reduction effects caused should be due to different capacities of the enzymes to degrade polysaccharides in the banana pulps during enzyme-substrate contact time. Similar effects on viscosity reduction though in juice not wine, had been earlier reported by Koffi *et al.* (1991) in their studies on viscosity reduction and prevention of browning in the preparation of clarified banana juice. Generally, wines obtained from Kayinja proved to be the cloudiest even after enzymatic action. This may be attributed to its high total soluble solids (24.5-27.1^0 Brix) in the juice after extraction as presented in Table 3.4 (a). Although the major component of TSS is sugar, the kind of elastic pulp observed during preparations for juice extraction from

Kayinja seem to indicate more colloidal (non-sugar component) material in Kayinja than in the other two cultivars. Olsen (2000) observed that colloids are formed by interaction of yeast glucans and grape pectins during the fermentation process. This may also be the case because in banana fermentations. The dissolved material (non-sugar) especially those of proteinaceous nature (witnessed as protein hazes in wine) may be responsible for the turbidity of the wine. It is also reported (Siebert *et al*., 1996; Siebert and Lynn, 1997; Ribéreau-Gayon *et al*., 2000) that tannins (found in bananas) could combine with the proteins by hydrogen bonds and hydrophobic interaction to induce haze in wines. The turbidity reduction results shown in Figure 4.1 (a), (b) and (c) caused by the enzymatic actions in the banana pulps did not show any significant difference in the enzyme treated wines obtained from the three banana cultivars. However, there was significant (p<0.05) difference between the enzyme treated and control wines in turbidity reduction.

Table 4.2: The effect of pectinolytic enzymes on turbidity levels in wines made from juices that were obtained from pulps of three banana cultivars**.**

Cultivar	Enzyme	Turbidity(NTU)
Bogoya(genotype AAA)	Rapidase X-press	7.07±0.16^{b}
	Rapidase CB	7.31±0.12^{b}
	Rapidase TF	4.17±0.07^{a}
	OE-Lallzyme	4.1±0.05^{a}
	Control	57.48±0.55^{cd}
Mbidde(genotype AAA-EA)	Rapidase X-press	9.21±0.05^{ab}
	Rapidase CB	9.96±0.08^{c}
	Rapidase TF	8.99±0.06^{ab}
	OE-Lallzyme	7.78±0.29^{b}
	Control	59.11±0.39^{d}
Kayinja(genotype ABB)	Rapidase X-press	11.26±0.12^{ac}
	Rapidase CB	12.67±0.44^{cb}
	Rapidase TF	11.2±0.05^{ac}
	OE-Lallzyme	9.59±0.14^{bc}
	Control	62.81±1.39^{dc}

Data are means ± SD of three experiments replicated.
[a]Different letters in the same column indicate significant differences (p<0.05)

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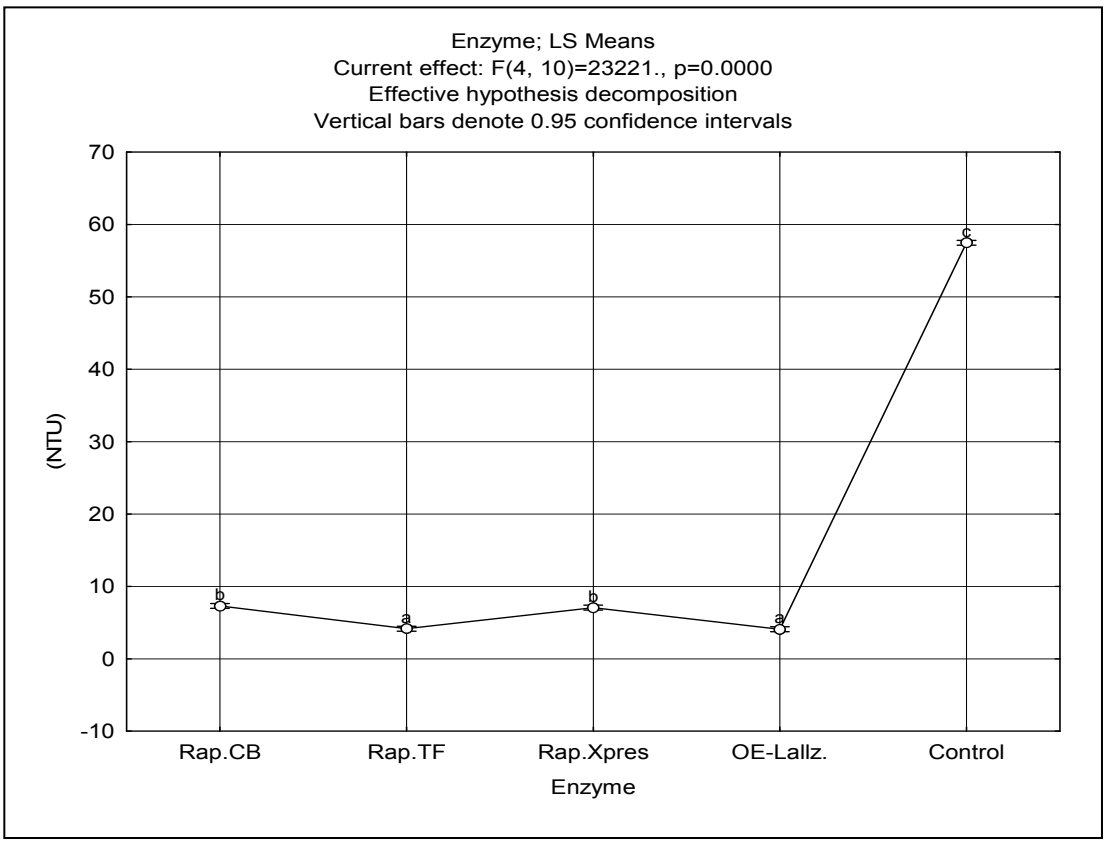

Figure 4.1 (a): Effect of enzymatic action on the turbidity of Bogoya banana wine.

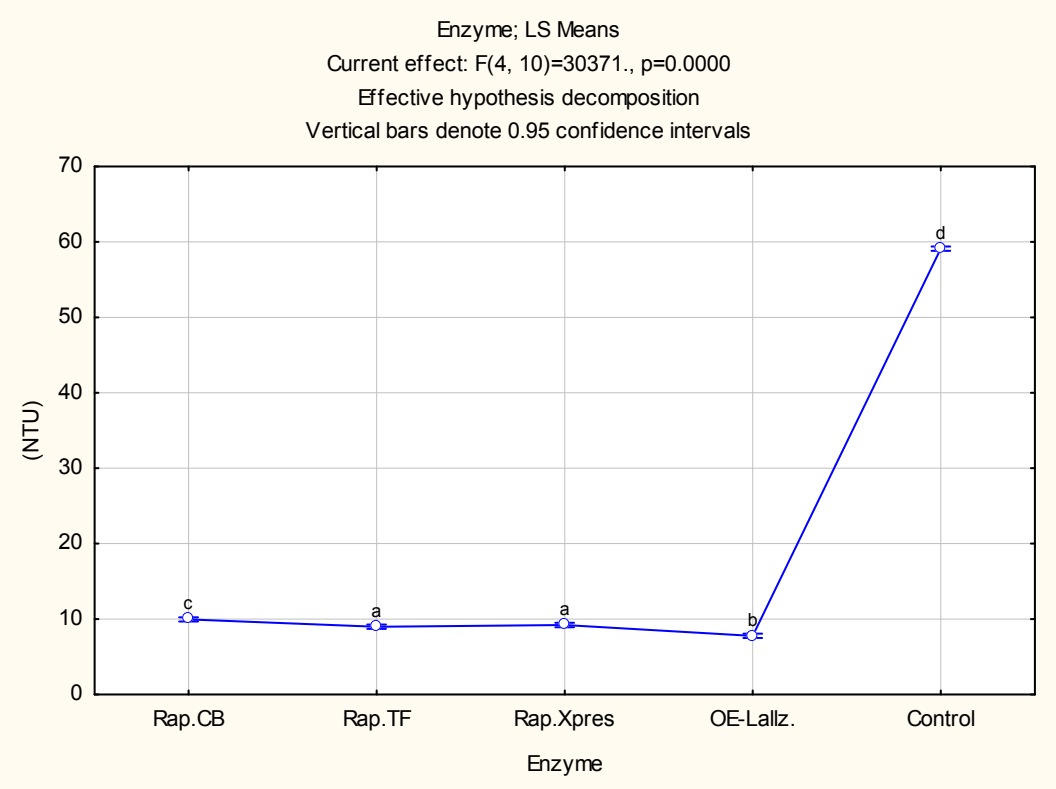

Figure 4.1 (b): Effect of enzymatic action on the turbidity of Mbidde wine.

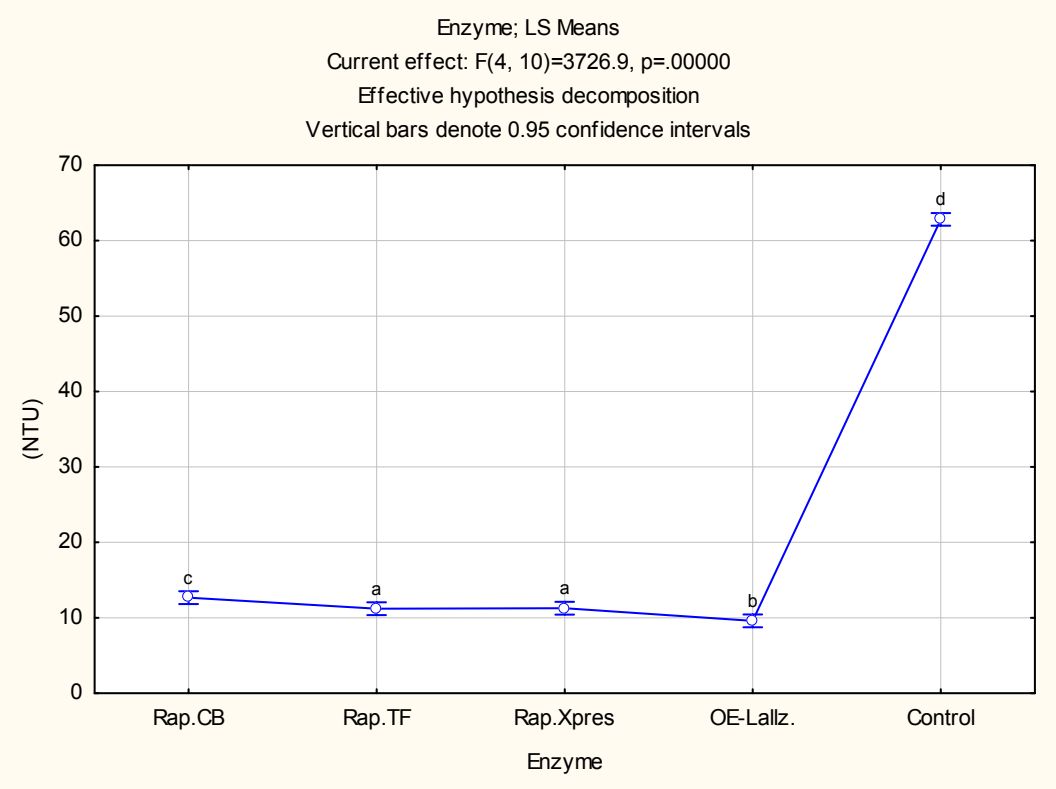

Figure 4.1 (c): Effect of enzymatic action on the turbidity of Kayinja wine.

The results of the effects caused by proteases applied to remove hazes from the banana wine are presented in Figure 4.2. Although there was a steady decrease in turbidity in all the samples including the control, there was a significant difference ($p<0.059$) in turbidity reduction after one and four weeks of enzyme treatment respectively. The decrease in turbidity in the control could be attributed to gravitational settling that continued to effect clarification of the young banana wine. It can be noted from the graph (Figure 4.2) that turbidity after one week in the Kayinja wine was recorded at approximately 47, 27.5 and 18 NTU in the control, Zumizyme and Papaine treated wines respectively. Turbidity after 4 weeks was at approximately 29, 18 and 10 NTU in the control, Zumizyme and papaine enzyme treated wines respectively. After 1 week, the wine turbidity had been lowered by approximately 11.3, 30.8 and 40.3 NTU from the initial wine turbidity (58.3 NTU) in control wine, Zumizyme and Papaine enzyme treated wines respectively. After 4 weeks, the wine turbidity had been lowered by about 18, 9.5 and 8 NTU in the control wine, Zumizyme and Papaine enzyme treated wines respectively. Papaine was more effective in settling the protein hazes in Kayinja wine than Zumizyme, with a significant difference ($p<0.05$).The rate of reducing turbidity from 58.3 NTU was greater in the first week (40.3 and 30.8 NTU in a week for Papaine and Zumizyme respectively) than the

subsequent 3 weeks period (8 and 9.5 NTU in 3 weeks for Papaine and Zumizyme respectively). The rate of turbidity reduction in the control for a week was 11.3 NTU and 18 NTU for the subsequent 3 weeks. The rate of turbidity decrease started slowing down after first week in enzyme treated wines more slowly than in the control wine. This may imply that the enzymes tend to loose their activity at a fast rate after the first few days of their specific action on the substrate. This may be an interesting aspect for future investigation.

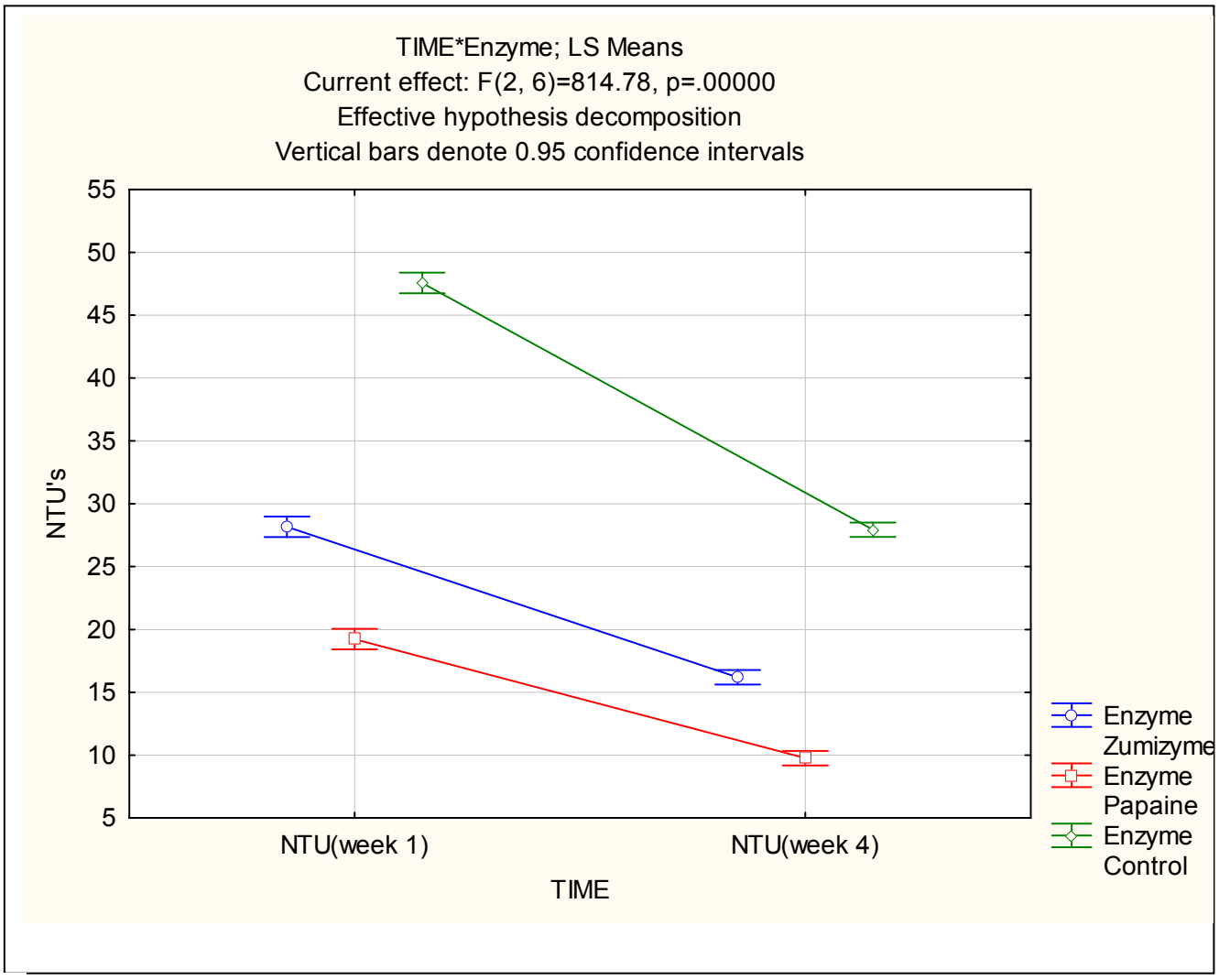

Figure 4.2: Effect of proteases on hazes in Kayinja banana wine at ambient temperature (18-22^{0}C).

4.3.2 Physicochemical characteristics of the banana wines

The results of the analysis of the physicochemical characteristics of the banana wines are presented in Table 4.3. The TSS of the banana juices ranging between 15.2 and 27.1^{0}Brix (Table 3.4a) was lowered to 4.2-5.8^{0}Brix in wines. Although there was slight but not significant *(p*>0.05) differences in TSS of wines made from juices that were obtained from enzyme treated pulps, the lowest TSS was observed in wines made from juice extracted from Rapidase X-press treated pulps in each cultivar. The pH was highest in the samples not treated with enzymes. The lowest pH of 3.2 was observed in the wine prepared from juice extracted from Rapidase CB enzyme treated pulp of the Mbidde cultivar. Kyamuhangire *et al.* (2002) reported that the average pH of juice extracted by the mechanical method was significantly higher than that of juice extracted by enzymatic method. These results of TSS in the banana wines also showed a similar trend with what the above researchers had noted, though again their observation was in banana juice. Uhlig (1998) reported that every enzyme requires a specific pH value for its optimal activity. This enzyme specificity is of prime importance when choosing an enzyme for industrial process. For maximum enzyme efficiency, enzyme with optimum activity above pH 3 must be chosen.

Titratable acidity (TA) was the highest in the control wine samples of all three cultivars, at 8.4 g/Lin Bogoya, 7.3 g/L in Mbidde and 6.16 g/L in Kayinja. The lowest TA was measured at 5.24g/L in wine made from juice extracted from Rapidase TF treated pulp in Kayinja. The TA concentrations can be attributed to the fact that the common acids in banana juice such as malic, citric, and succinic acids (Kyamuhangire *et al.*, 2002) are weak acids which produce few ions per mole of compound in the juice solution during ionization process. In acceptable levels, these acids enhance flavour of the product and increase palatability of the product. The TA in wines can also play a preservative role by creating an unfavourable environment for different microorganisms (bacteria and yeasts) that could cause spoilage. Volatile acidity (VA) was detected at the highest level in the control wine samples from all three cultivars. The lowest VA levels were obtained in Mbidde and Kayinja at 0.5 g/L with Rapidase TF, Rapidase CB and OE-Lallzyme treated wines. However, the acetic acid as a volatile compound found in the wine distillate (Table 4.6) was found higher in wines made from juices extracted from enzyme-treated pulps (except in Rapidase CB treated pulp) compare to the control wines. Since VA is reported (Radler, 1993) to

be mostly composed of acetic acid (>90%), and it is further reported (Zoecklein *et al*., 1995) that acetic acid has a threshold of 0.7 to 1.1 g/L, the VA results (ranging from 0.5 to 0.8 g/L) obtained from these banana wines do fall below or within the threshold of the grape wines acetic acid concentrations reported above. This VA concentration range found in banana wines in this study was not too high to make the banana wine unacceptable.

Table 4.3: Physicochemical characteristics of wines obtained from the three banana cultivars

Cultivar/Parameter	Control	Enzymes used in wine preparation			
		Rapidase TF	Rapidase CB	Rapidase X-press	OE-Lallzyme
Bogoya (*Musa*, AAA)					
TSS	5.4	4.4	4.9	4.2	4.7
pH	4.01	3.95	3.81	3.92	3.89
TA (g/L)	8.4	7.2	7.6	7.06	7.3
VA (g/L)	0.74	0.61	0.63	0.59	0.62
Reducing sugars (g/L)	6.8	0.61	0.52	0.58	0.68
Turbidity (NTU)	8.4	4.06	4.09	3.31	5.2
Alcohol (% v/v)	6.4	8.3	8.1	8.2	8.4
Mbidde (*Musa*, AAA-EA)					
TSS	5.8	5.5	5.5	4.5	5.3
pH	3.86	3.73	3.2	3.57	3.59
TA (g/L)	7.3	5.5	6.9	6.4	5.9
VA (g/L)	0.8	0.6	0.5	0.6	0.6
Reducing sugars (g/L)	3.4	0.75	2	1.8	1.3
Turbidity (NTU)	9.02	6.1	6.3	4.08	5.82
Alcohol (% v/v)	6.8	8.4	8.6	9.2	8.5
Kayinja (*Musa*, ABB)					
TSS	5.6	5.1	5.4	4.2	5.2
pH	3.96	3.92	3.93	3.91	3.88
TA (g/L)	6.16	5.24	5.62	5.8	5.42
VA (g/L)	0.7	0.5	0.6	0.6	0.5
Reducing sugars (g/L)	8.2	2.4	3.1	2.7	1.8
Turbidity (NTU)	11.4	7.2	8.1	5.6	6.3
Alcohol (%v/v)	9.3	14.4	13.6	14.6	13.4

Data are means of three experiments replicated.

The highest reducing sugars levels were found in the control wines from all three cultivars. The lowest reducing sugars levels were in Bogoya wines. Higher alcohol yields were also obtained from the wines prepared from juices extracted from enzyme-treated banana pulps compare to the control wines. The highest alcohol yield, of 14.6% (v/v) was obtained from Kayinja wine, made from juice that was extracted from a pulp treated with Rapidase X-press enzyme. This high alcohol

content is associated with the higher sugar content (in TSS of>26^{0}Brix) in the Kayinja banana cultivar. The lowest alcohol yield of 6.4%v/v in the Bogoya control wine is related to the low sugar content (15.2°Brix). The enzyme treated samples is not much higher in this cultivar and may be due to the commercial enzymes tested not being optimal for this cultivar. The pectinolytic enzymes can cause more sugar to be released for the yeast to utilize for alcohol production during enzymatic degradation of pectin. The control wines made with no enzymes added from the Bogoya and Kayinja cultivars contained significantly less alcohol than the enzyme-treated wines as shown in Figures 4.3 (a), (b) and (c). The wine turbidity was significantly reduced in the wines produced from enzyme-treated pulps. The most effective enzyme in turbidity reduction was Rapidase X-press used in preparations of Bogoya wine, followed by Rapidase TF. This has proved that that Rapidase X-press does not only have the capacity to effectively increase juice yields, but also to reduce turbidity, and thus emerged as the most effective enzyme for wine clarification among this group of commercial enzymes tested. This is in agreement with other research findings (Jackson & Badrie, 2002; Mabesa *et al*., 1989), which have shown that different commercial enzymes enhance banana pulp digestion, produce better yields and improve the clarity of juice and wine made from (*Musa acuminata*) bananas.

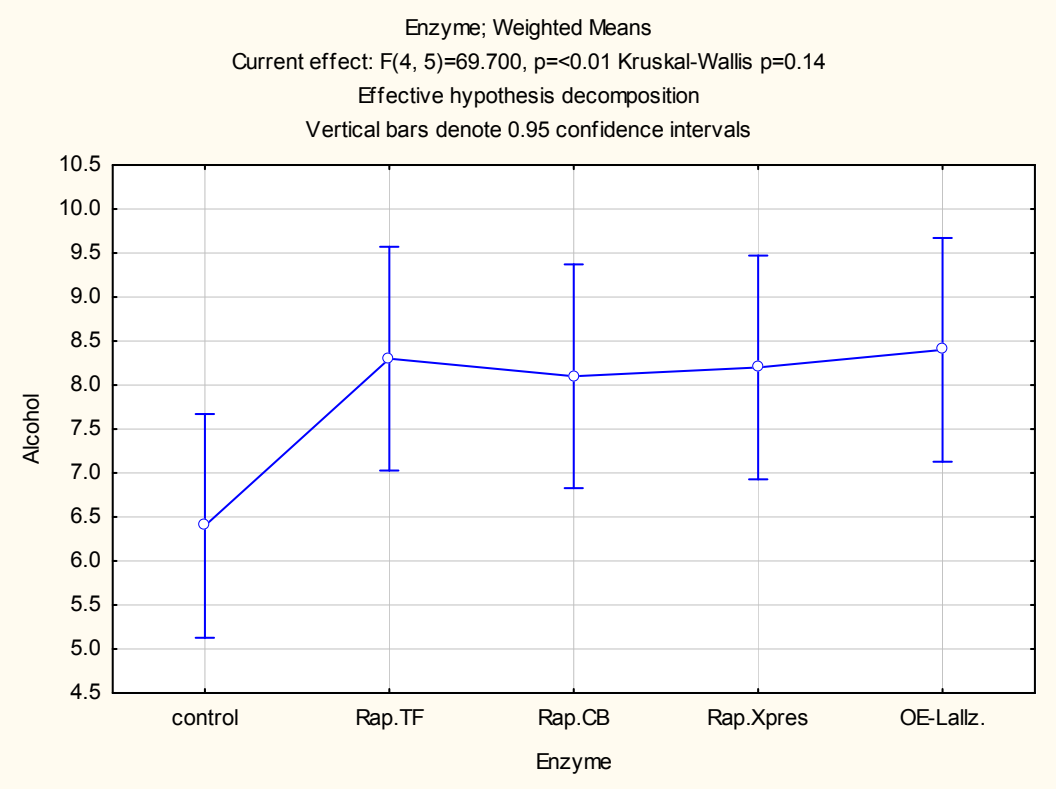

Figure 4.3 (a): Alcohol (%v/v) produced in *Bogoya* wines treated with commercial enzymes

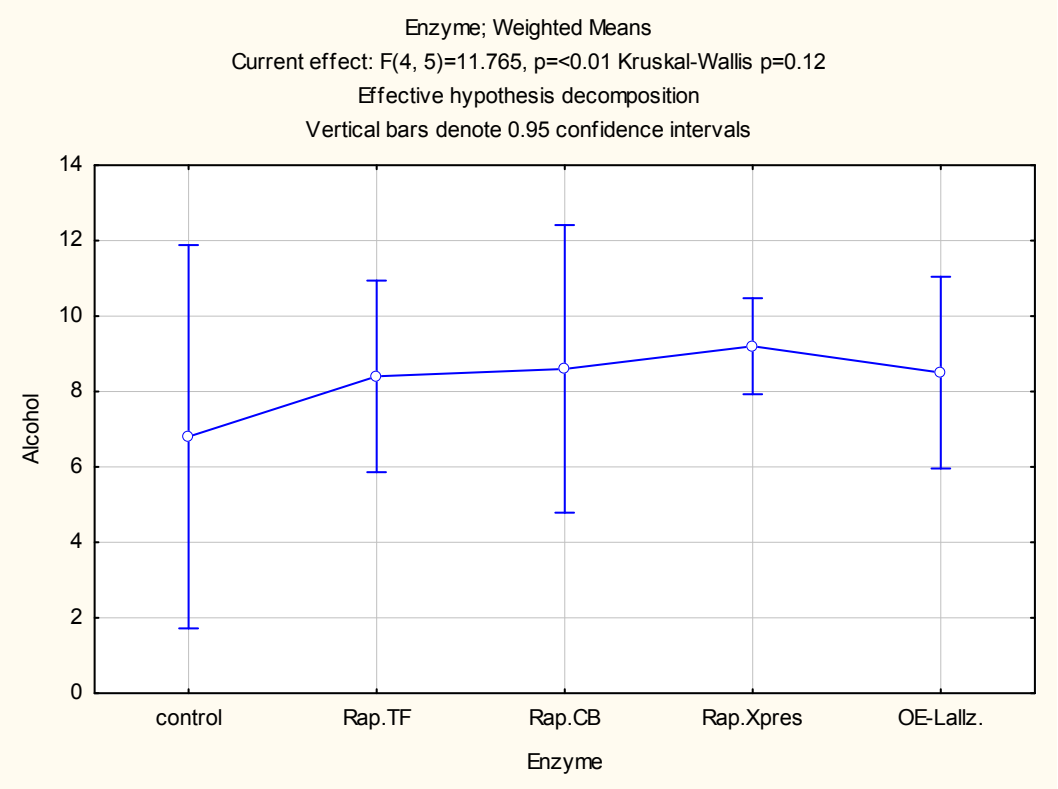

Figure 4.3 (b): Alcohol (%v/v) produced in *Mbidde* wines treated with commercial enzymes

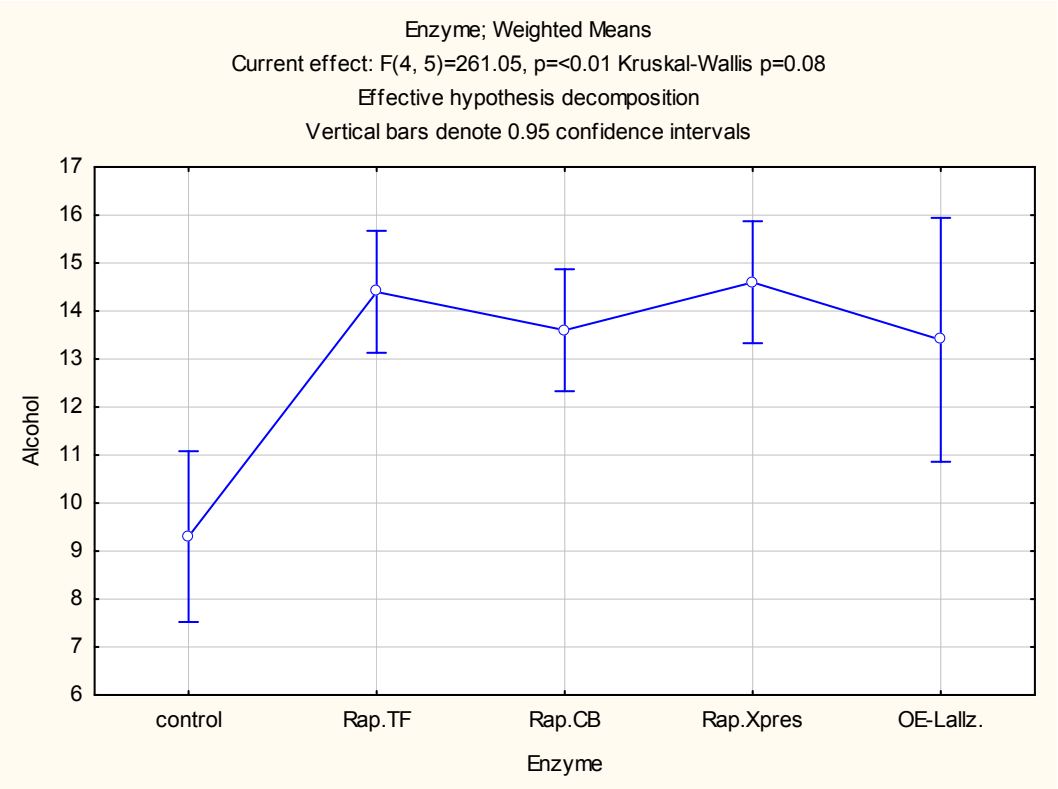

Figure 4.3 (c): Alcohol (%v/v) produced in *Kayinja* wines treated with commercial enzymes

4.3.3: Sensory evaluation of banana wines

Sensory evaluation of banana wines which were processed by applying different pectinolytic enzymes were assessed using both the ranking and descriptive analysis methods. The results are presented below.

Ranked sensory attributes

The results of the sensory evaluation are presented in Figures 4.4 (a), (b), (c) and (d). The mean values of the sensory profiles of the attributes assessed are plotted in radar graphs. There was no significant difference ($p>0.05$) between the attributes evaluated by the assessors in wines treated with the different pectinolytic enzymes as per ANOVA test results (Appendix 4).

Quality is a composite response to the sensory properties of wine based on the assessor's expectations and hence an individual response is based on preferences and experiences (Noble *et al*., 1984). However, the wines that were given the highest scores for overall acceptability were from Kayinja juice. Kayinja juice was analysed with the highest total soluble solids averaging 26.5°Brix. The high soluble solids gave Kayinja wine the most preferred taste and ultimately its wines were the most accepted wines. Nevertheless all the banana wines assessed were rated with an overall acceptance of at least 61% (an average score of 5.5/9 on a nine- point hedonic scale). The acceptability of banana wines had earlier been confirmed by other researchers (Akingbala *et al*., 1994; Joshi *et al*., 2000) when wines were made from overripe bananas. Neubeck (1975) reported that use of commercial pectic enzymes in wine preparations produce wines with a fruitier flavour and bouquet. The results of this study show that these banana wines were of an acceptable level according to the sensory evaluation carried out by the members of the taste panel. However, to improve the wines in terms of palatability, it may be necessary to do further manipulations which may include blending of say a wine and another wine prior to aging in non-experimental wine preparations.

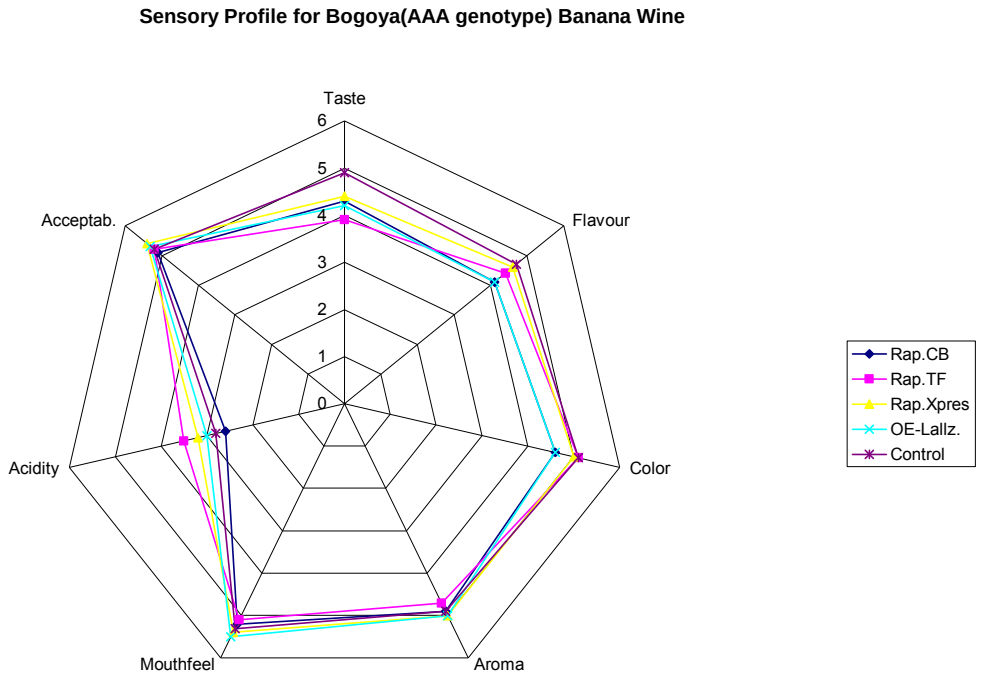

Figure 4.4 (a): Spider chart representing different sensory profiles for *Bogoya* wines obtained from enzyme treated pulps and their control

In Bogoya wine, Figure 4.4(a), flavour, taste and colour attributes were scored highest on the nine-point hedonic scale in the control wine. On the other hand, flavour and colour scored lowest in the wine made from juice that was extracted from OE-Lallzyme treated pulp while taste was assessed lowest in the wine made from juice extracted from Rapidase TF treated pulp. The aroma, mouthfeel and acceptability were scored highest in the wine prepared from juices obtained from OE-Lallzyme treated pulp. Aroma and mouthfeel were assessed lowest in wine prepared from juice obtained from Rapidase TF treated pulp while acceptability scored lowest in wine made from Rapidase CB treated pulp. All enzyme treated wines and the control made from the Bogoya (*Musa*, AAA genotype) cultivar did not show a significant difference ($p>0.05$) in the acceptability (Appendix 4). Acidity was highest in wines made from juices extracted from Rapidase TF and lowest in Rapidase CB treated pulps respectively. The acidity reported by the panel does not agree to the TA presented in Table 4.3. This may be due to some other attribute that caused the wine to be perceived as more acid.

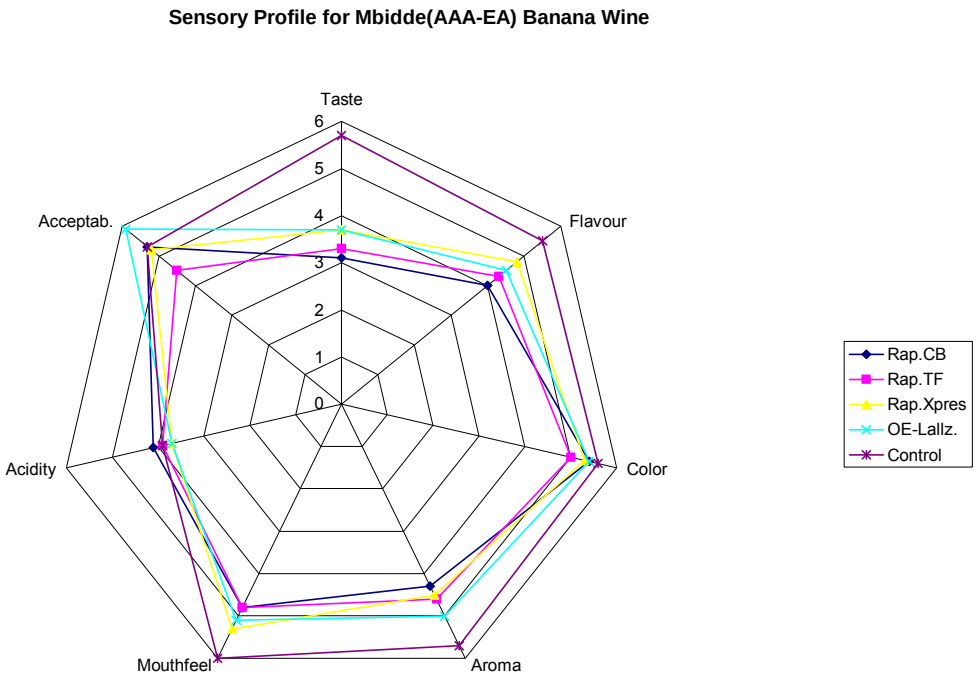

Figure 4.4 (b): Spider chart representing different sensory profiles for *Mbidde* wines obtained from enzyme treated pulps and their control

In Mbidde wine, Figure 4.4(b), flavour, taste, colour, aroma and mouthfeel attributes were scored highest on the nine-point hedonic scale in the control wine. Favour, taste and aroma were scored lowest in the Rapidase CB treated wine whereas colour and mouthfeel were assessed lowest in the Rapidase TF treated wine. The acceptability were scored highest in the OE-Lallzyme treated wine and lowest in Rapidase TF treated wine. Acidity was highest in Rapidase CB and lowest in OE-Lallzyme treated wines respectively.

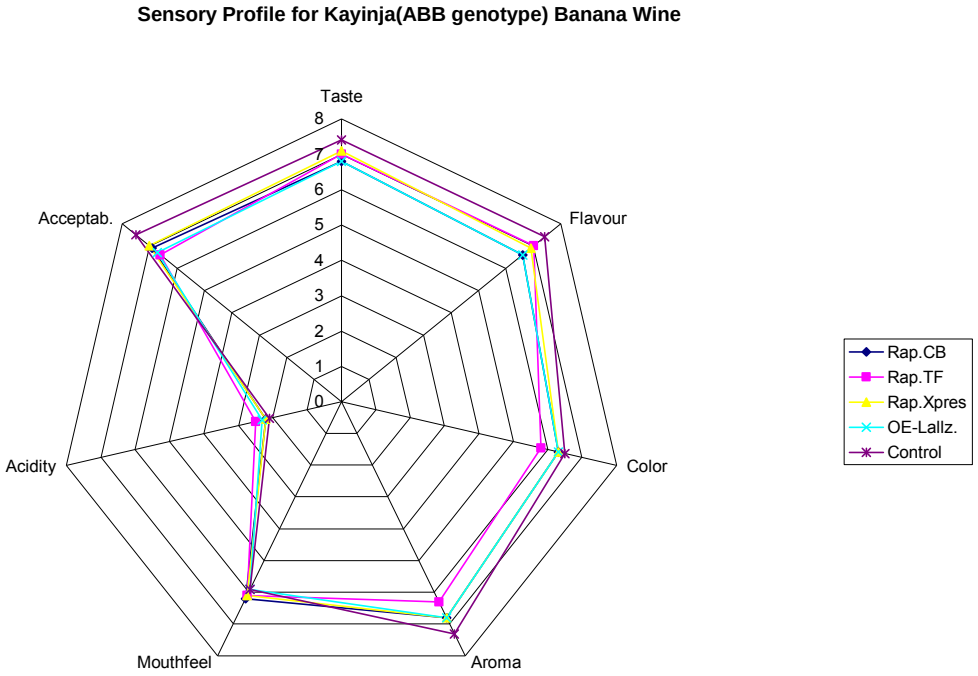

Figure 4.4 (c): Spider chart representing different sensory profiles for Kayinja wines obtained from enzyme treated pulps and their control

In Kayinja wine, Figure 4.4 (c), flavour, taste, colour, aroma and acceptability attributes were scored highest on the nine-point hedonic scale in the control wine sample. Flavour, taste and mouthfeel were scored lowest in the OE-Lallzyme treated wine whereas colour, aroma and acceptability were assessed lowest in the Rapidase TF treated wines. All enzyme treated wines and the control of Kayinja (*Musa*, ABB genotype) cultivar did not show a significant difference ($p>0.05$) in the mouthfeel attribute (Appendix 4). Acidity was highest in Rapidase TF treated wine and lowest in the control wine.

The means of the attributes for the combination of the 3 cultivars for each enzyme is presented in Figure 4.4 d. The panel put the overall acceptability of the wines at a score ranging between 5.3 and 6.0 on the nine-point hedonic scale. Taste scored lowest (4.6/9) in wines prepared from juices extracted from Rapidase CB treated banana pulps and highest (6.8/9) in wines prepared from juices extracted from the untreated (control) pulps. Flavour also scored least (4.8/9) in wines made from juices that were extracted from Rapidase CB treated pulps and highest in wines made from juices extracted from the control pulps. Colour scored lowest (5.3/9) in wines prepared from juices extracted from Rapidase TF treated pulps and highest (5.8/9) in the wines prepared from the control banana pulps. Aroma also scored lowest (5.1/9)

in wines made from juices extracted from Rapidase TF treated pulps and highest (5.9/9) in wines made from the untreated pulps. Mouthfeel also like aroma scored lowest (5.1/9) in wines prepared from juices extracted from Rapidase TF treated pulps and highest (5.7/9) in wines prepared from juices extracted from the control pulps. Acidity was assessed least (3.0/9) in wines made from juices extracted from Rapidase CB treated pulps and most (3.5/9) in wines made from juices extracted from Rapidase TF treated pulps. The overall acceptability of the banana wines scored lowest (5.3/9) in wines prepared from juices that were extracted from Rapidase TF treated pulps and highest (6.0/9) in wines prepared from juices that were extracted from the untreated(control) banana pulps. The effects caused by enzymatic treatment of the banana pulps may be either positive(in case of lowering the acidity and mouthfeel) or negative (in case of lowering taste and aroma) depending on the quality of wine preferred by the consumers. Ultimately, though changes noted in the banana wines prepared from pulps treated with pectinolytic commercial enzymes were slightly different ($p>0.05$) (see Appendix 4), the wines made from the juices extracted from the control pulps scored highest (6/9), that is about 66.7% acceptance. Vilanova *et al.* (2000) had reported that with reference to wines obtained from must supplemented with commercial pectolytic enzymes, as far as typicality is concerned, these wines display aromas that are less typical or not typical at all due to the release of terpenes and esters, a consequence of the action of some of the commercial enzymes. The results of sensory evaluation by panel seem to support what had been noted by the above researchers. The members of the sensory panel appreciated the banana wines with a percentage of 61% (mean score: 5.5/9) of acceptability. Such effects (presumably negative) brought about by enzymatic processing may be overcome by blending juices from treated and untreated banana pulps during preparations of non-experimental wines and quality products may be obtained for economic purposes as we acknowledge the positive aspects (e.g. increased yields, turbidity reduction) behind enzymatic processing of banana beverages

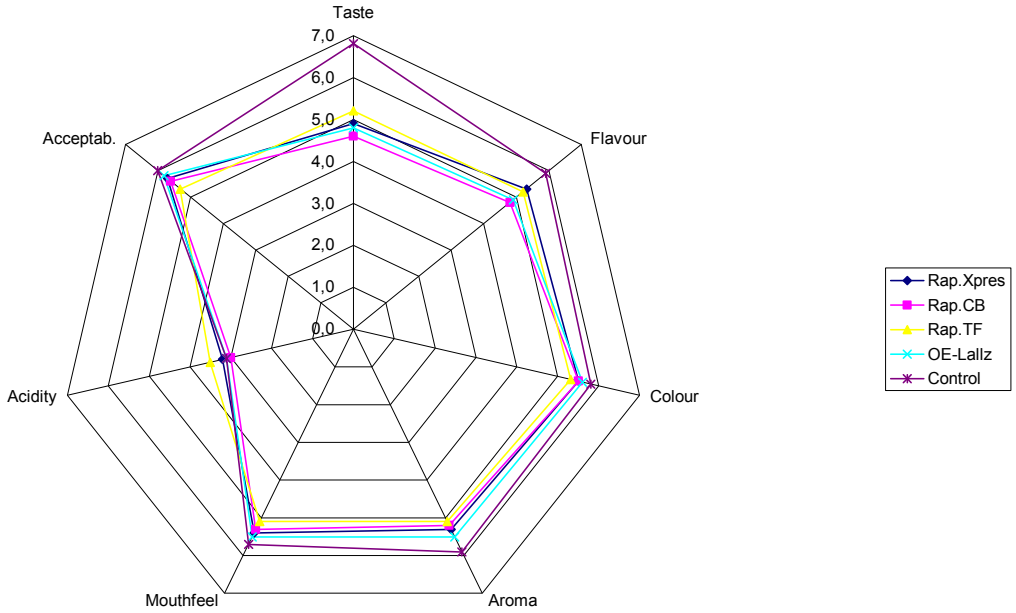

Figure 4.4(d): Spider chart representing combined sensory profiles for Kayinja, Mbidde and Bogoya wines obtained from enzyme treated pulps and their controls. Comparisons were made by a panel of 20 tasters and data are described using radar graphs following statistical statistcal evaluation.

Description of banana wine in flavour profile analysis

The results of wine flavour for three different wines obtained from three banana cultivars that were treated with the same enzyme and those for five wines from same cultivar but treated with different pectinolytic commercial enzymes and their control are presented in Table 4.4 (a) and (b) respectively.

Table 4.4 (a): The Flavour Profiles of Wines obtained from juices of three Banana Cultivars treated with Enzyme Rapidase X-press.

Banana Cultivar Wines	Wine Flavour Descriptors
Bogoya(*Musa* AAA genotype) wine	sweet, acidic, lightly sour, dry
Mbidde(*Musa* AAA-EA genotype) wine	very sweet, very fruity, lightly acidic.
Kayinja (*Musa* ABB genotype) wine	sour, sweet, fruity, astringent, aromatic

Table 4.4 (b): The Flavour Profile of Mbidde (*Musa* AAA-EA genotype) Wines made from juices obtained from pulps treated with different enzymes.

Banana Wines	Wine Flavour Descriptors
Rapidase CB treated wine	Sweet, fruity, acidic
Rapidase TF treated wine	Fruity, astringent, sweet
Rapidase X-press treated wine	Acidic, astringent, dry
OE- Lallzyme treated wine	Sweet, fruity, smooth
Control wine	Sweet, light body, sour

The wines obtained from the three banana cultivars (Figure 4.4 a) had in common the attribute of sweetness. Bogoya and Mbidde wines tasted acidic. Bogoya wine to some extent was sour an attribute that was also found in Kayinja wine. The acidity and sourness in the wine is associated with malic acid and acetic acid respectively that are present in wines. Such organic acids in bananas have been reported by other researchers (Sadler and Murphy, 1998; Kyamuhangire *et al*., 2002) and also in grape wine (Bartowsky and Henschke, 2004). Mbidde and Kayinja wines were found to be fruity. This fruity nature is a characteristic aroma of the wine, a result of esters produced by yeast during fermentation as it was previously reported by Margalit (1997). However, Bogoya wine was described as dry whereas Kayinja wine was astringent. Astringency in Kayinja (*Musa*, ABB genotype) juice was earlier reported by Kyamuhangire *et al*. (2002). This astringency occurs as result of tannins in the fruit that give a harsh mouthfeel. The various Mbidde (*Musa* AAA-EA, genotype) wines which were processed from banana pulp treated with different commercial enzymes were tasted sweet in the three wines prepared from juices obtained from enzyme treated pulps and control, except in the wine obtained from juice extracted from pulp treated with Rapidase X-press. The banana wines which tasted fruity were prepared from juices obtained from pulps treated with Rapidase CB, Rapidase TF and OE-Lallzyme enzymes. Acidity was detected in wines made from juices extracted from pulps treated with Rapidase CB and Rapidase X-press. Some astringency (a harsh mouthfeel) was detected in wine made from juices extracted from pulps treated with Rapidase TF and Rapidase X-press enzymes. The sensory characteristic of astringency in bananas is usually associated with unripe and immature fruit. This could have resulted from the last fingers on the banana bunches that normally are not as mature as the other banana fingers. Astringency could have also occurred as a result of some banana fingers not being so ripe especially those located along the heap sides during the ripening process since the highest temperature was found in the banana heap centre. Wine prepared from juice extracted from OE-Lallzyme

treated pulp tasted smooth while the Mbidde control wine was described as light bodied. Flavour is a wine's most important distinguishing characteristic and the bouquet of a wine is determined by the presence of a well-balanced ratio of flavour compounds and metabolites (Lambrechts and Pretorius, 2000). A wine's flavour could in its widest sense, be said to be the overall sensory impression of both aroma and taste compounds, and may therefore incorporate the more measurable aspects of acidity, sweetness, alcohol strength, fizziness, astringency and bitterness (Robinson, 1994). The members of the sensory panel appreciated (mean score: 5.5/9) the banana wine flavour and recommended it for market trials. That implied that the attributes assessed in the wine flavour profiles were of acceptable levels to the panel and could as well be acceptable to consumers.

4.3.4 Microbiological analysis

Microbiological analysis of the banana wines after one week, one month and three months after bottling indicated that no microbial spoilage occurred (data not shown). Acetic acid bacteria (AAB) which are gram-negative and lactic acid bacteria (LAB) which are gram-positive were not found in the treated wines after the periods of one week, one month and three months. Coli forms and yeasts were also not found in the wine after the same period of ageing. The absence of micro organisms in the wine was attributed to the effectiveness of the SO_2 used as an antimicrobial agent at 50 ppm. Inhibitory conditions to bacterial growth were also contributed by the alcohol level in the banana wine especially for the coliforms which are easily inhibited by alcohol and the good hygienic manufacturing practices (GMP) that were used during winemaking, including successful anaerobic conditions with no oxygen ingress into the fermenting biomass. After three months, the wine was regarded as mature and no microbial spoilage was detected in the wines.

Usually, in case of bacterial contamination with oxygen ingress into wine vessels or bottles, a distinctive ring at the interface between the wine and headspace in the bottle neck (Bartowsky and Henschke, 2004) may be seen. Such contamination is characterized with unpleasant smell of the wine. Similar contamination occurred in one of the fermented wine samples with that kind of ring growth in the bottle neck after one month on shelf and the banana wine had unpleasant smell. It was established that, that contamination was from acetic acid bacteria after gram-staining analysis. Such bacterial growth is favoured by oxygen ingress into the bottle after

sealing when the wine bottles are stored in a vertical position on the shelf (Bartowsky and Henschke, 2004).

4.3.5 The volatile compounds in banana wine distillates

The Kayinja banana wine distillates that were analysed as described in Materials and Methods (i.e. by gas chromatography) showed various volatile compounds in milligrams per litre (mg/L). Results are presented in Table 4.5.

The results showed significantly higher levels of methanol in the banana wines distillates than in grape wine distillates. Methanol was highest (953.1 mg/L) in the wine treated with Rapidase X-press and lowest (563.8 mg/L) in the control respectively. It is generally noted that methanol is not produced by alcoholic fermentation, but is primarily derived from hydrolysis of naturally occurring pectins and higher methanol has been reported when pectolytic enzymes are added to musts or pomace (Amerine *et al.*, 1980). The pectin content of 0.5-1% occurring in bananas with pectin methyl esterase enzyme naturally found in bananas plus commercial pectinases applied to degrade pectin should be responsible for the methanol production. A wide survey on reported methanol content in wines around the world (Gnekow and Ough, 1976) showed that the average concentration of methanol in white wines is 60 mg/L (40-120 mg/L range), and 150 mg/L in red wines 120-250 mg/L range). The legal limit of methanol in the US is 1000 mg/L and although methanol is toxic, its concentrations in wines do not provide any risk (Margalit, 1997). The toxicity of methanol as a fatal dose *(Merck Index*) is (100-250) ml per person or 340 mg/kg body weight (Christensen, 1973). Methanol is metabolised in the body like ethanol (although at a slower rate) and thus taking into account methanol content in wine, of 100 mg/L, and using the more strict toxicity value of 340 mg/kg, a person whose weight is approximately 70 kg, has to drink about 200 litres of wine(!) in order to be effected by methanol (Margalit, 1997). It is further reported (Zoecklein, 1999) that wines made from fruit other than grapes may especially be high in methanol content because of their higher relative pectin levels. Distillates produced from plum and apricots have been reported to have methanol contents of 2,000 to 5000 mg/L (Woidich and Pfannhauser, 2007). Earlier on, Vilanova *et al*. (2000) had reported that with reference to wines obtained from must supplemented with commercial pectolytic enzymes, methanol levels increased twofold due to the activity of pectin methyl esterases on pectin, and as far as typicality is concerned, these wines display

aromas that are less typical or not typical at all due to the release of terpenes and esters, a consequence of the action of some of the commercial enzymes. The results obtained in this study on methanol levels in the wine samples agree with those of the above mentioned researchers because wines made from juices extracted from enzyme treated pulps showed higher methanol content than in the control wine. The safety of the banana wines that were prepared in this study falls below the legal limits of US methanol levels discussed above. And thus, this implies that the banana wine produced is safe in terms of methanol level concentrations that were established.

It was established that in almost all the Kayinja banana wine samples processed using enzymes, the volatile compounds concentrations were higher than in their control wines except in the case of acetoin and butyric acid that did not fall in the same trend. Ethyl acetate concentration in the banana wine was much higher than its concentrations in grape wines. It is stated (Du Toit and Pretorius, 2000) that grape wines with concentrations of 200 mg/L ethyl acetate are regarded as spoiled. To establish the concentrations of ethyl acetate in banana wines that probably would render it spoiled may require further research. The results obtained in this study seem to agree with those earlier reported by Vilanova as far as enzymes role in increasing the volatiles in wine is concerned. Although not all cultivars of bananas and grapes have been compared in terms of volatile compounds, the results available (Appendix 6 and Table 4.5) seem to suggest that grape wines have more different volatile compounds than the Kayinja banana wines or the GC method that was used is not optimal for banana wine distillates.

With a comparison of volatile compounds in grape wines and banana wines, a number of common volatile compounds were found in both grape and banana wines. These included methanol, butanol, ethyl acetate, isoamyl acetate, ethyl butyrate, ethyl caprylate, ethyl caprate, ethyl lactate, diethyl succinate, acetoin, hexanol, isobutyric acid, butyric acid, isovaleric acid and decanoic acid. The volatile compounds that were found higher in banana wines than in the grape wines included methanol, butanol, ethylacetate, isoamyl acetate, ethyl butyrate, ethyl lactate, diethyl succinate, acetoin, isobutyric acid, butyric acid, isovaleric acid and decanoic acid. Ethyl caprylate was found lower in banana wines than in the grape wines although it was not detected in the control banana wine and in the wine made from juice obtained from banana pulp treated with enzyme Rapidase TF.

The volatile compounds are responsible for the flavour (taste and aroma) of the wine. However, a quality product should always have these volatiles at acceptable levels. Acetic acid has a threshold value of 0.7 to 1.1g/L depending on the style of wine and above these values it becomes objectionable (Zoecklein *et al*., 1995). The occurrence and accumulation of the volatile compounds in wines are results of various reactions that take place in metabolic pathways. During the metabolism of sugars, lipids and nitrogenous substances by yeast, many various metabolites are formed. Many of these metabolites are produced in relatively small quantities, in comparison to ethanol and carbon dioxide (primary products) and are referred to as "secondary metabolites (for a detailed review on how the volatile compounds are formed, see Delfini and Formica, 2001). The major secondary metabolites are glycerol, pyruvic acid and acetaldehyde. The other secondary metabolites are formed from pyruvic acid via oxidative decarboxylation to acetyl-Co-A, or from the reduction of acetaldehyde, or from a combination of both.

Table 4.5: Volatile compounds found in banana (*Musa* ABB genotype) wine

	Enzyme				
Compound(mg/L)	**Rap.TF**	**Rap.CB**	**Rap. X-press**	**OE-Lallz.**	**Control**
Ethyl acetate	866.88	762.7	783.36	1015.69	590.08
Methanol	923.78	812.29	953.1	682.83	563.82
Ethyl butyrate	7.6	3.38	8.9	75.19	0.86
n-Propanol	65.55	39.39	62	63.08	12.15
Isobutanol	117.4	96.89	121.75	109.81	nd
Isoamylacetate	7.24	5.37	1.85	7.02	1.13
n-Butanol	9.63	7.4	9.16	8.19	1.86
Isoamylalcohol	269.19	196.8	231.9	249.05	42.62
Ethyl hexanoate	0.52	nd	0.65	0.53	nd
Hexyl acetate	nd	nd	nd	nd	nd
Acetoin	140.65	373.8	132.2	106.51	244.48
Ethyl lactate	21.51	42.9	120.14	17.91	25.54
Hexanol	21.94	1.75	1.15	1.63	0.38
Ethyl caprylate	nd	0.2	0.35	0.14	nd
Acetic acid	431.91	130	442.94	913.84	311.68
Isobutyric acid	20.84	27.59	10.81	21.48	19.53
Butyric acid	38.42	17.78	41.16	310.94	146.69
Ethyl caprate	0.26	0.14	0.14	nd	nd
Isovaleric acid	11.04	18.67	9.05	10.06	10.2
Diethyl succinate	1.43	1.69	2.14	1.75	1.55
n-Valeric acid	nd	nd	nd	0.19	nd
2-Phenylethyl acetate	0.16	0.23	0.49	0.2	0.19
Hexanoic acid	1.11	1.14	1.42	0.98	0.85
2-Phenylethanol	6.24	8.97	16.66	9.01	8.7
Octanoic acid	1	0.82	2.32	0.72	0.62
Decanoic acid	0.5	0.48	1.1	0.49	nd

In the Table, "nd" denotes "not detected", (Rap.TF=Rapidase TF; Rap.CB=Rapidase CB; Rap. Xpres=Rapidase X-press; OE.Lallz =OE-Lallzyme)

The results of this study have that a number of volatile compounds such as methanol; acetoin and hexanol (see Table 4.5 & Appendix 6) are in much higher concentrations in the banana wine than in the grape wine. The other issue is that some volatile compounds like some esters (e.g. ethyl succinate and ethyl pyruvate) which occur in grape wine may not occur in banana wine and vice versa. The commercial pectinolytic enzymes used in this study like other commercial pectinolytic enzymes (in different trade names) used by other researchers aided in processing banana juice and wine and this is an issue of economic importance. Olsen (2000) acknowledged the role of pectinolytic enzymes by stating that today; they play a key role in modern fruit juice technologies and further added enzymes make it possible to achieve good process economics. Pectinolytic enzymes are a prerequisite for obtaining clear and stable juices and high juice and wine yields.

Enzymatic treatment of banana pulp significantly influenced certain effects during banana wine processing. The aspects which were very significant included the capacity of the applied pectinolytic enzymes to reduce turbidity and an increase in reducing sugars in the wines made from juices obtained from enzyme treated banana pulps compared to their controls. The enzyme treatment of banana pulp resulted in higher alcohol (% v/v) yields after fermentation and this should be related to the high TSS in the juice as a result of enzymes added. This may be a positive effect depending upon the levels of alcohol that may be desired by the final wine consumer. However, it was also found that wines made from juices obtained from the enzyme treated pulps had higher methanol and other volatile compounds levels in the distillates compared to their controls. Aked and Kyamuhangire (1996) had reported that excess banana beer (fermented banana juice with shorter shelf life) is further processed into a spirit called *waragi* through a process of distillation. This may lead to higher methanol levels (Table 4.5) in distillates and may be toxic. The methanol levels in the wines produced in this study do not put the banana wine consumer at risk as it has been explained above that such levels are not toxic unless too much wine is consumed. Wine prepared from bananas had been reported by different researchers and authors. Kundu *et al.* (1976) reported that good quality wine was obtained from ripe and over-ripe banana (*Musa peradisiaca*) and details on the banana wine that was made were reviewed in section 2.2.1. Jackson and Badrie (2002) reported that standard-quality wines with percentage alcohol of 14.57-15.84%,TA as 0.59-0.76 % malic acid, VA of 0.06-0.08g/100ml, free SO_2 of 88.75-

109.75mg l^{-1} and with low microbial counts (<10 cfu ml^{-1}) were produced from Lacatan variety bananas. Akingbala *et al.* (1992) after preparing wine from over-ripe bananas (*Musa acuminata*), concluded that over-ripe bananas can be salvaged by processing into a wine. Lewis (2002) reported of a banana grower in Australia who also turned bananas into wine. Different processing techniques have been used by various processors to try and make safe and quality banana wine (see section 2.2.1).

In this study an acceptable banana wines with no microbial counts, (pH 3.2-4.01, TA 5.24-8.4 g/Las malic acid, VA 0.5-0.8 g/L, reducing sugars 0.52 -3.2g/L, wine turbidity 3.31-11.4 NTU and alcohol content in the range of 6.4-14.6%) were made from juices obtained from enzyme treated and untreated pulps of three different cultivars grown in tropical and sub-tropical climates.

4.4 CONCLUSIONS

The enzymes tested showed a significant difference ($p<0.05$) in turbidity. Haze stabilisation was significantly effected by the action of proteases in the wines, particularly after one week of incubation with added proteases. The most acceptable wine from the three cultivars was obtained from Kayinja. This study has elucidated that over-ripe banana (*Musa* AAA genotype) juice can be exploited for (conventional) banana winemaking.

This information should be helpful to prospective banana wine processors to profitably utilize the so called “rejected” banana fruits from the market and produce acceptable wines and enable them produce safe banana based distillates.

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CHAPTER 5

RESEARCH RESULTS: CHARACTERISATION OF BANANA WINE FERMENTED WITH RECOMBINANT WINE YEAST STRAINS

Abstract

Commercial enzyme preparations are applied in the process of winemaking to improve wine processing and wine quality. In this study, three recombinant wine yeast strains that had been developed to improve wine processing were compared with regard to their ability to produce banana wine of an acceptable quality. The recombinant wine yeast strains contain genes coding for enzymes able to degrade different polysaccharides (glucan, xylan, pectin and starch). The recombinant strains were able to induce higher wine yields from the fermented banana pulp *vis-à-vis* the control. The wines obtained with the recombinant strains and the untransformed control did not show significant differences ($p>0.05$) in their physicochemical parameters. The highest wine yield obtained from the pulp which was treated with genetically modified yeast (recombinant yeast strain) secreting glucanase and xylanase enzymes (plasmid pDLG39) was 69.5% v/w prepared from Bogoya pulp. This wine yield was higher than the control yield (65.9± 0.07) by an increase of 5.5% v/w. However, the wine from Kayinja pulp which was treated with genetically modified yeast secreting pectinase enzyme(plasmid pPPK) yielded wine (60.3±0.28% v/w) with 35% increase compared to the wine (44.7±0.49) obtained with the control yeast. The recombinant yeast strains compared in this work secreted enzymes playing a similar role to that of the previously studied pectinolytic commercial enzymes. Therefore, such a recombinant yeast strain could be used in banana wine fermentations as an alternative to commercial enzyme preparations.

Key words: Recombinant yeast, pectinase, glucanase, xylanase, amylase, wine

5.1 INTRODUCTION

Biotechnology in general, and genetic engineering in particular, have in the recent few decades enabled gene manipulation and enhanced technologies in applied agricultural and food biotechnology research. These advances in DNA technology have made it possible to clone genes with traits of interest to the winemaker and to manipulate organisms' cells to overproduce the desired protein. During the last 18 years, several attempts to construct genetically engineered yeast strains have been published, and very interesting improvements in the wine-making process or the quality of the wine obtained have been reported, including improved primary and secondary flavors, malic acid decarboxylation by yeast, increased resveratrol, lactic acid, or glycerol contents, and improved survival properties (Barros *et al*., 2000; Dequin *et al*., 1999; Ganga *et al*., 1999; Gil and Valles, 2001; Gonzales-Candelas *et al*., 2000; Lilly *et al*., 2000; Perez-Torrado *et al*., 2002; Remize *et al*., 1999; Smit *et al*., 2003; Vilanova *et al*., 2000; Volschenk *et al*., 2001). Many enzymes have been expressed in manipulated micro-organisms at levels of 10 to 100 times higher than in their natural host cells (Kresse, 1995; 2001). The degradation of structural polysaccharides by carbohydrases can result in an improvement in juice yield, clarification and filterability during winemaking (Colagrande *et al*., 1994; Haight and Gump, 1994; Van Rensburg and Pretorius, 2000). *Saccharomyces cerevisiae* lacks the ability to produce extra cellular depolymerising enzymes that can efficiently liberate fermentable sugars from abundant, polysaccharide-rich substrates, and this limits *S. cerevisiae* to a narrow range of carbohydrates. A number of targets being set for the genetic improvement of wine yeasts. These targets include increasing the efficiency of the fermentation progress, the processing of wine and control of microbial spoilage, in addition to enhancement of wholesomeness and sensory quality (Henschke, 1997; Snow, 1983; Pretorius and Van der Westhuizen, 1991 Pretorius, 1999, 2000, 2001, 2002). Therefore, the exploitation of recombinant yeast strains with the ability to utilise such complex polysaccharides can aid in the cost-effective production of heterologous proteins of biological interest (Gundllapalli Moses *et al*., 2002). As an alternative strategy to the addition of costly enzyme preparations that often contain unwanted contaminating or side activities, wine yeasts are being developed to secrete proteolytic and polysaccharolytic enzymes that would remove haze-forming proteins and filter-clogging polysaccharides during fermentation. To this end, the overexpression of several bacterial, fungal and yeast

genes has resulted in proteolytic, pectinolytic, glucanolytic and xylanolytic wine yeast strains (Querol and Ramon 1996; Pretorius, 1997; Van Rensburg and Pretorius, 2000; Gognies *et al*., 2001; Laing and Pretorius, 1993; Pérez-Gonzélez *et al.*, 1993; Van Rensburg *et al*., 1998). To comply with the ever-increasing demands of modern winemakers and consumers for the best quality wine at every price point, it is also inevitable that novel enzymes will be designed for specific purposes and then tailored through protein engineering technologies (Van Rensburg and Pretorius, 2000).

In the previous chapters, we indicated that we were able to produce a stable banana wine of acceptable quality by applying different polysaccharide-degrading commercial enzymes during the winemaking process. As far as we know, this will be the first attempt to apply recombinant wine yeast strains for improved processing in banana wine production, with the aim also to produce a stable banana wine of acceptable quality.

5.2 MATERIALS AND METHODS

5.2.1 Banana cultivars used

The two types of bananas used were Kayinja, which was obtained from Uganda, and Williams of the same Bogoya (*Gros Michel*) genotype, was bought from “Fruit and Veg City” in Stellenbosch, South Africa. The bananas in each set of experiments were ripened to stage 8 (yellow, speckled brown) of ripeness under special conditions, with temperatures ranging between 28 and 33˚C.

5.2.2 Yeast strains and plasmids

The control wine yeast strain was *Saccharomyces cerevisiae* VIN13 (from Anchor Yeast Biotechnologies, South Africa). VIN13 is also the parent yeast that had been manipulated into the recombinant strains. The genotypes and phenotypes of the recombinant yeast used in this study are summarised in Table 5.1.

Table 5.1: Recombinant wine yeast strains used in this study

Plasmid	Phenotype	Genotype	Reference
pPPK	Pectinolytic	*URA3::ADH1$_P$-MFα1$_S$-pele-TRP5$_T$ ADH1$_P$-MFα1$_S$-peh1-TRP5$_T$*	Louw *et al*., 2006
pDLG31	Amylolytic	*URA3::PGK1$_P$-LKA1-PGK1$_T$*	Gundllapalli Moses *et al*., 2002
pDLG39	Glucanolytic Xylanolytic	ILV2:: *ADH1$_P$-MFα1$_S$-end1-TRP5$_T$ YG100$_P$-XYN2-ADH2$_T$*	Louw *et al*., 2006

5.2.3 Culturing media and inoculation

The yeast was grown in yeast peptone dextrose (YPD) broth (1% yeast extract, 2% peptone and 2% dextrose). To inoculate the cultures, 10 ml of YPD medium was inoculated with a single selected colony of the desired yeast strains and incubated overnight at 30°C. After incubation, 1 ml of the yeast culture was introduced into 200 ml YPD medium. The yeast cells were allowed to grow at a temperature of 30°C for three to four hours on a shaker. Then, after determining the yeast cell growth in the medium, the cells were centrifuged at 5000 rpm for 5 min with a g-force of 2200. The pellets formed in the centrifuge tubes were re-suspended in 30 ml of distilled water. The banana pulp was inoculated at 2 x 10^6 cells per ml.

5.2.4 Microvinification experiments

The fermentation assays were carried out using two different banana cultivars. The over-ripe bananas at stage 8 (yellow, speckled brown) were hand peeled and pulped using a motorised mixer. The banana pulp was measured in kilograms to determine the correct ratio of processing aids. After pulping, 50 ppm of SO_2 was added to the pulp. During these pre-fermentation operations, the pectinaceous nature (the thickness) of the banana pulp proved problematic (colloidal and viscous) to handle in pre-fermentation operations and the pulp was thus diluted with water in a ratio of 3:1 to reduce the viscosity of the pulp and make more liquid (juice-water mixture) available in the fermenting biomass. Diammonium phosphate (DAP) was applied to the pulp at a rate of 0.7 g/kg to supplement the nitrogen required in alcoholic fermentation. The transformed recombinant (DNA) yeast strains and the untransformed (VIN13, control) yeast were used to inoculate the banana pulps in duplicates. The inoculated pulps were allowed to ferment at room temperature. The progress of the fermentation was monitored by measuring the weight loss per day until a constant weight was attained for three days. The Clinitest (Bayer Corporation,

USA) method where reagent tablets are added in wine solution to estimate reducing sugars (in percentages) was used after no further weight loss was observed for three days. The wine yields in percentages volume by weight (%v/w) was calculated by dividing the wine volume (litres) by the weight (kilograms) of the banana pulp inoculated with the volume of water used for dilution excluded.

5.2.5 Wine stabilisation and filtration

After complete fermentation, banana wine was extracted from the pulp, stabilised and filtered through 0.3 µm filter pads treated with citric acid, with diatomaceous earth as a filter aid and at a pressure of 50-150 kPa in a batch pressure filter. Various parameters were then analysed (see Table 5.3) in the sampled batches.

5.2.6 Physicochemical analyses of banana wine

The pH of the wine was measured by using a glass calomel electrode pH meter (Cole Parmer, Vernon Hills, IL, USA). Together with titratable acidity, the pH was determined again, this time automatically with a 20:702 SM Titrino (Metrohm). The soluble solids (°Balling) in the wine were determined using a hydrometer as outlined by Iland *et al*. (2000). Reducing sugars (g/L) were analysed by means of the Rebelein method, described by Iland *et al*. (2000). Volatile acidity (VA) was determined by distillation in a B&M Scientific Markham still. The free sulphur dioxide (SO_2) of the wine was determined by an iodine titration technique, using the Ripper method as outlined by Iland *et al*. (2000). The turbidity of the wine was measured using a Hack turbidimeter and expressed in nephelometric turbidity units (NTU), while the alcohol content was measured in percentage volume by volume (%v/v) by two methods, namely alcoholometry and ebulliometry as outlined by Iland *et al*. (2000).

5.3 RESULTS AND DISCUSSIONS

5.3.1 Wine fermentation

All the various fermentations were completed on average in 15 days. All the wines that were produced contained less than 3 g/L reducing sugars and can be considered

as dry wines. No significant difference in fermentation performances were observed between the control yeast (VIN13) and the recombinant (DNA) yeast strains.

5.3.2 Wine yields

The pulps inoculated with genetically modified yeast yielded banana wine as presented in Table 5.2. The wine yields were analysed statistically and the results are presented in Figure 5.1 (a) and (b). The total bulk of Kayinja bananas that was obtained from Uganda had been peeled and yielded 60% of the fruits weight (kg), and the rest 40% discarded in the form of peelings (waste). The highest wine yield (60.3 ± 0.28%) obtained in Kayinja was from the pulp treated with yeast strain VIN13-pPPK, which phenotypically (Table 5.1) had pectinolytic activity and yielded a percentage increase of 34.9% more wine than the control. The *Erwinia chrysanthemi* pectate lyase gene (*pelE)* and *E.ycarotovora* polygalacturonase gene (*peh1*) were each inserted between a yeast expression-secretion cassette and a yeast gene terminator in the plasmid pPPK formation. Pulps treated with strains VIN13-pDLG31 (transformed with amylase coding gene) and VIN13-pDLG39 (transformed with glucanase and xylanase coding genes) yielded 21.7% and 20.1% wine increases respectively more than the control (untreated pulp) from the Kayinja cultivar. This elucidated that the main problematic polysaccharide in Kayinja banana fruit is pectin compared to glucan and xylan and the most appropriate enzymes to degrade Kayinja pulp would be pectinases.

In the case of Bogoya cultivar, the wine yield increases were approximately 1%, 1.4% and 5.5% with VIN13-pPPK, VIN13-pDLG31 and VIN13-pDLG39 treated banana pulps respectively. The Kayinja banana cultivar showed a significant (p<0.05) difference in the wine yields obtained from the pulps treated with recombinant yeast strains compare to the control. Bogoya cultivar showed only slight differences in wine yields between the recombinant yeast fermented pulps and the control. The difference in wine yields between the two types of bananas of different genotypes seems to indicate that the enzymes in the transformed yeast work better in Kayinja than in Bogoya. The polysaccharides in Bogoya seem to be more readily degraded at ripeness than in Kayinja as earlier discussed in chapter three. Even with no exogenous enzymes added (see control), the Bogoya cultivar yielded wine (65.9%) close to the yields from recombinant yeast treated pulps. The endogenous enzymes

in Bogoya seem to be very effective with little influence in wine yield effected by supplementary exogenous enzymes (particularly pectic and amylolytic) for further degradation of the polysaccharides in the pulp to yield wine. However, the yeast strain transformed with glucanase and xylanase coding genes (VIN13-pDLG39) showed significant difference ($p<0.05$) in Bogoya. This suggests that glucan and xylan (part of polysaccharides complex) in Bogoya require exogenous enzymes to supplement the endogenous enzymatic activities.

Viquez *et al*. (1981) reported that the addition of pectinase enzymes in the preparation of wines served to reduce viscosity, facilitated pressing of fermented solids and in so doing increased the yield. The results of wine yields in this study are in agreement with what Viquez *et al*. (1981) had earlier reported particularly in Kayinja cultivar where wine yields (34.9%) were very significant.

Table 5.2: Wine yields from banana cultivars fermented with recombinant yeast strains.

Yeast	Cultivar	Yield(%v/w) Mean ± SD
pPPK	Kayinja	60.3 ± 0.28^{c}
	Bogoya	66.4 ± 0.52^{d}
pDLG31	Kayinja	54.4 ± 0.07^{b}
	Bogoya	66.8 ± 0.43^{d}
pDLG39	Kayinja	54.0 ± 2.12^{b}
	Bogoya	69.5 ± 1.06^{e}
VIN13 (Control)	Kayinja	44.7 ± 0.49^{a}
	Bogoya	65.9 ± 0.07^{d}

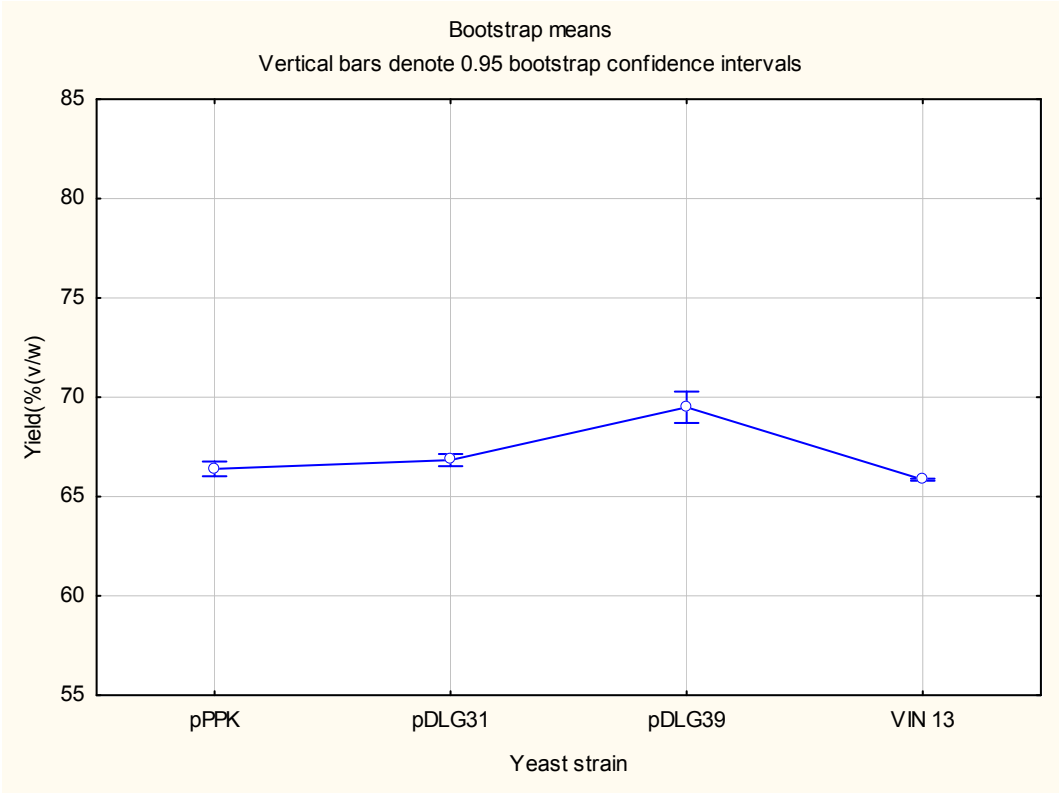

Figure 5.1 (a): Wine yields obtained from Bogoya (*Musa* AAA, genotype) pulps.

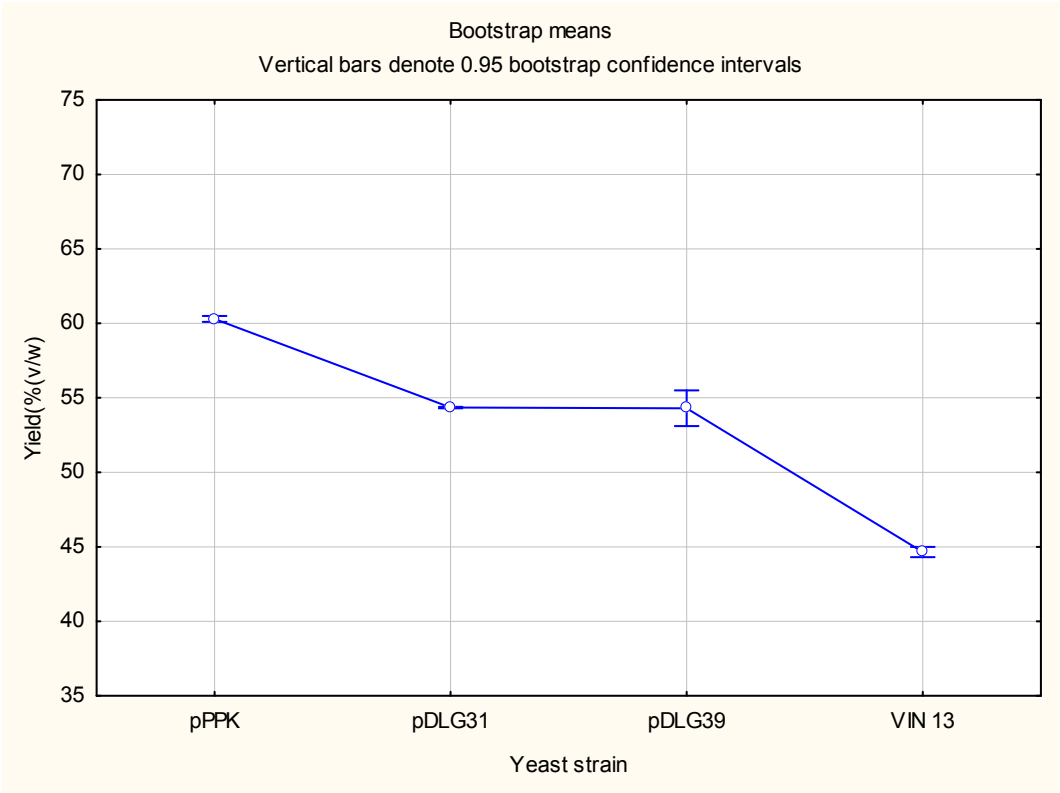

Figure 5.1 (b): Wine yields obtained from Kayinja (*Musa* ABB genotype) pulps.

The wine yields are compared in Figure 5.2. Although the yields only showed big differences in Kayinja cultivar, the transformed wine yeast strains gave higher results than the control in both cultivars. Therefore, it can be concluded that the transformed yeast strains are suitable for juice and wine extraction from over-ripe bananas.

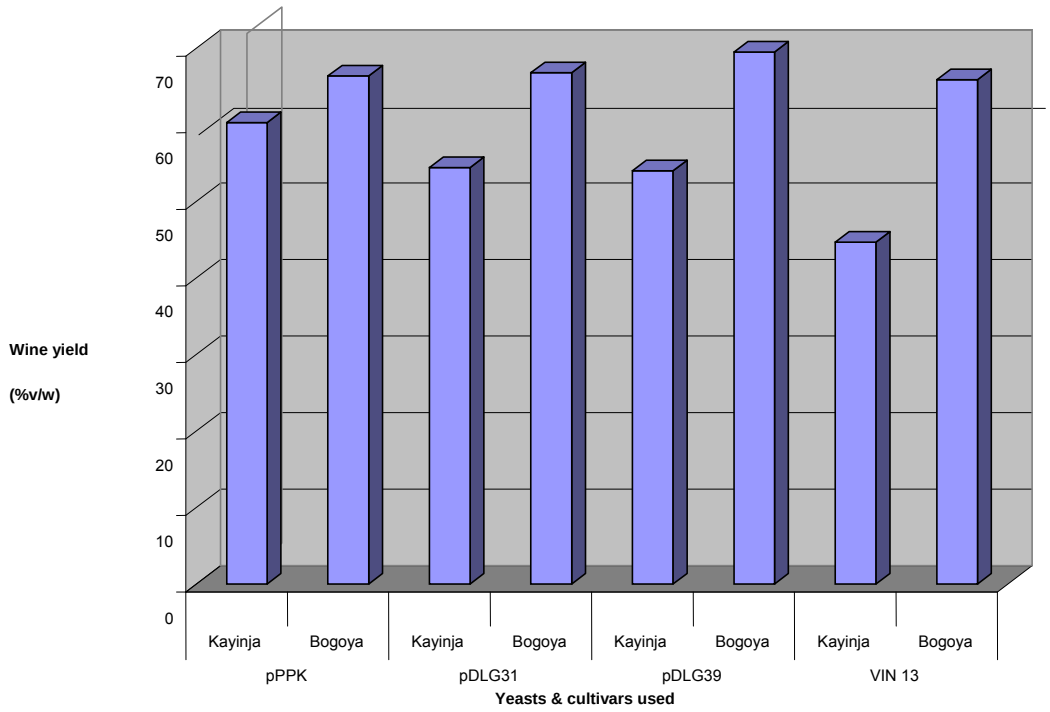

Figure 5.2: Wine yields (%v/w) from Kayinja and Bogoya banana pulps fermented with recombinant yeast strains

5.3.3 Physicochemical analysis of banana wine

The physicochemical properties of the banana wines made using recombinant yeast strains are presented in Table 5.3.

°Brix of juice obtained from the pulp

The sugar content in terms of the total soluble solids of the juice was measured at 26.4°Brix in Kayinja and 15°Brix in Bogoya before dilution of pulp with water. The degrees Brix in Kayinja could potentially have produced an alcohol percentage of 15.05% (calculated as, 26.4 x 0.57=15.05), but practically gave a highest level of 9.9% (v/v). The 0.57 as conversion factor was selected from the range (0.55-0.60)

reported as experimental value measured in various studies on wines (Marsh, 1958; Ough and Amerine, 1963; Ough and Singleton, 1968; Jones and Ough, 1985). The final alcohol percentage obtained can be attributed to the dilution made which reduced the brix from 26.4 to 17.6^{0}Brix by the dilution ratio of 3:1. Secondly, whereas 90-95% of TSS is sugar (Margalit, 1997; Iland *et al*., 2000), not all the sugar (about 8%) is converted into ethanol. Margalit *et al*. (1997) stated that abut 5% of the sugar is consumed to produce by-products (glycerol, succinic acid, lactic acid, 2,3-butanediol,acetic acid, etcetera), about 2.5% is consumed by the yeast as a carbon source and about 0.5% are left over as unfermented residual sugars. And this explains why the actual alcohol content is always less than potential alcohol (based on theoretical calculations). The degrees brix in Bogoya were low (15.7°Brix) on average. This could have resulted either from the bananas not being harvested at full maturity, or the banana stools grown under conditions that limited the sugar (TSS) formation in the fruits, for example, bananas grown under shade or in a cold climate. The low brix in Bogoya juice naturally gave a lower alcohol content (6.1-7.7%) compared to Kayinja (8.5-9.9%) (Table 5.3) in this study.

Table 5.3: Physicochemical characteristics of banana wine fermented with recombinant (DNA) yeast strains.

Cultivar/Parameter	**Yeast plasmid/strain**			**Control**
Bogoya (AAA)	pPPK	pDLG31	pDLG 39	VIN 13
TSS	5.2	5.4	5.1	6.7
pH	3.62	3.7	3.7	3.8
TA (g/L)	6.7	5.6	6.47	5.1
VA (g/L)	0.66	0.47	0.64	0.58
Reducing sugars (g/L)	1.1	0.83	1.4	1.7
SO_2 ppm	36	38	38	45
Alcohol (%v/v)	7.3	7.65	7.6	6.1
Turbidity (NTU)	14.8	17.5	14.8	43.9
Kayinja(ABB)				
TSS	5.75	5.45	5.2	7.15
pH	3.93	3.95	3.84	4
TA (g/L)	5.59	4.44	4.76	4.55
VA (g/L)	0.55	0.57	0.59	0.46
Reducing sugars (g/L)	1.06	0.39	0.3	2.34
SO_2 ppm	32	34	37	42
Alcohol (%v/v)	9.4	9.8	9.9	8.5
Turbidity (NTU)	22.5	25.9	22.21	51.95

Turbidity of the Wines

Whereas most of the parameters did not show significant differences ($p>0.05$), the turbidity levels (NTU) remained significantly higher ($p<0.05$) in the control (VIN13). This shows that the transformed yeast strains effectively used their enzymatic activities to play a similar role as that of the commercial enzymes and that they can act as substitutes for that purpose.

The fining and clarification of wine often include expensive and laborious practices that generate large volumes of lees for disposal, thereby causing a loss of wine and removing aroma and flavour compounds from the wine that remains. To minimize these disadvantages, an increasing spectrum of relatively expensive commercial enzyme preparations (proteases, pectinases, glucanases, xylanases and arabinofuranosidases) are frequently added to the grape must and wine.As an alternative to such preparations, which often contain unwanted contaminating or side activities,wine yeasts are being developed to secrete proteolytic and polysaccharolytic enzymes that would remove haze-forming proteins and filter-clogging polysaccharides respectively. To this end, the overexpression of several bacterial,fungal and yeast genes has resulted in proteolytic,pectinolytic,glucanolytic and xylanolytic wine yeast strains (Querol and Ramon 1996; Pretorius, 1997; Van Rensburg and Pretorius, 2000; Gognies *et al*., 2001; Laing and Pretorius, 1993; Pérez-Gonzélez *et al*., 1993; Van Rensburg *et al*., 1998). The results on turbidity in this study are in support of what the above researchers had noted and such means of improving processing efficiency in bananas should be exploited to greater heights.

In Figure 5.3 (a) and (b) it was shown how effective the recombinant yeast strains affected the turbidity in both cultivars compared to the control fermentation. Amylases and arabanase hydrolyse starch and araban in fruit juices respectively, thereby preventing potential turbidity in packaged juices and wines due to precipitation of such polymers (Uhlig, 1998). The same role on turbidity has been affected according to our results in Figure 5.3 (a) and (b). Also according to Sreekantiah (1975), the application of enzymes in the clarification of fruit juices and fruit wines aids in the partial or complete hydrolysis of the suspended starches, proteins and pectins. It appears that the amylases in their major role of hyrolysing starch, they also caused significant ($p<0.05$) turbidity reduction in the banana wine.

Alcohol content in the wines

It was also elucidated that the alcoholic yield was higher when fermentation took place with the recombinant yeast in comparison to the control VIN13 yeast, as shown in Figures 5.4 (a) and (b) respectively. A comparison of the different recombinant yeasts shows that VIN13-pDLG39, which produces glucanolytic and xylanolytic enzymatic activities, was the best in influencing alcoholic fermentation. This highest alcohol content was obtained in Kayinja. Kayinja has higher sugars than Bogoya as earlier discussed in chapter three due to its high TSS (about 24-27^{0}Brix). When some of these polysaccharides are degraded, more sugars are released that can be converted to alcohol by the yeast and in this particular case glucans and xylans were degraded.

Wine analyses

The pH of the wines was higher in controls than in the wines obtained from enzyme treated banana pulps in both banana cultivars. The TA was more (except in Kayinja treated with pDLG31) in recombinant yeast inoculated pulps. The VA in the wines was not higher in the wines obtained from enzyme-treated pulps except with VIN13-PDLG31 which had VA of 0.47 (g/L). It is difficult to explain why TA and VA were less than in the control wines when the recombinant yeast with the amylolytic-enzymatic activity was used. Perhaps the degraded starch affected the TA and VA in those wines. The resultant wines showed VA levels below the maximum of 0.7g/L allowed in grape wines. A high level of VA, which is an indication of microbial activity and by spoilage bacteria such LAB and AAB, may also have detrimental effects upon the sensory quality of the wines. The reducing sugars remained the highest (Table 5.3) in the control wines in both cultivars. This means that recombinant yeast utilise the sugars more effective, which is difficult to explain since the control yeast is exactly the same except the one or two gene differences. The SO_2 was higher in the control wines from the two cultivars. The use of the enzyme-secreting genetically modified yeasts improved the processing of the banana wines and displayed positive aspects, wine yield was improved, more alcohol was produced and the wine clarity was better compared to the control wines.

The GM yeast strains showed almost the same effect on turbidity. However, in both Bogoya and Kayinja, though not very significant, the wine obtained from pDLG31

treated pulp, which had the amylolytic activity had the highest values (that is 17.5 and 25.9 NTU respectively) compared to other wines obtained from the enzyme-treated banana pulps. The yeast strains transformed with pectinase, glucanse and xylanase showed approximately the same levels, i.e.about 15 NTU in Bogoya and 22.5 NTU in Kayinja. It may be surprising that amylases had the same effect on turbidity as the pectinase, glucanase and xylanase enzymes. It is well known that enzymes are ideal for breaking down soluble compounds responsible for high viscosity in upstream and downstream processes. Reaction efficiency, specific action, the ability to work under mild conditions and a high degree of purification and standardization all make enzymes ideal for the starch industry. One example of this application is in sweetener production where alpha-amylases reduce the viscosity of gelatinized starch. The thick gel can be transformed into a liquid that flows like water (for details on reduction of viscosity, see Olsen, 2000). Since ripe bananas contain 1-4% starch, similar effects could have been caused amylolytic enzyme originating from the genetically modified yeast (VIN13-pDLG31) which was directly inoculated into pulp leading to viscosity and turbidity reduction. Such turbidity reduction by amylase and arabanase enzymes had been reported by Uhlig (1998).

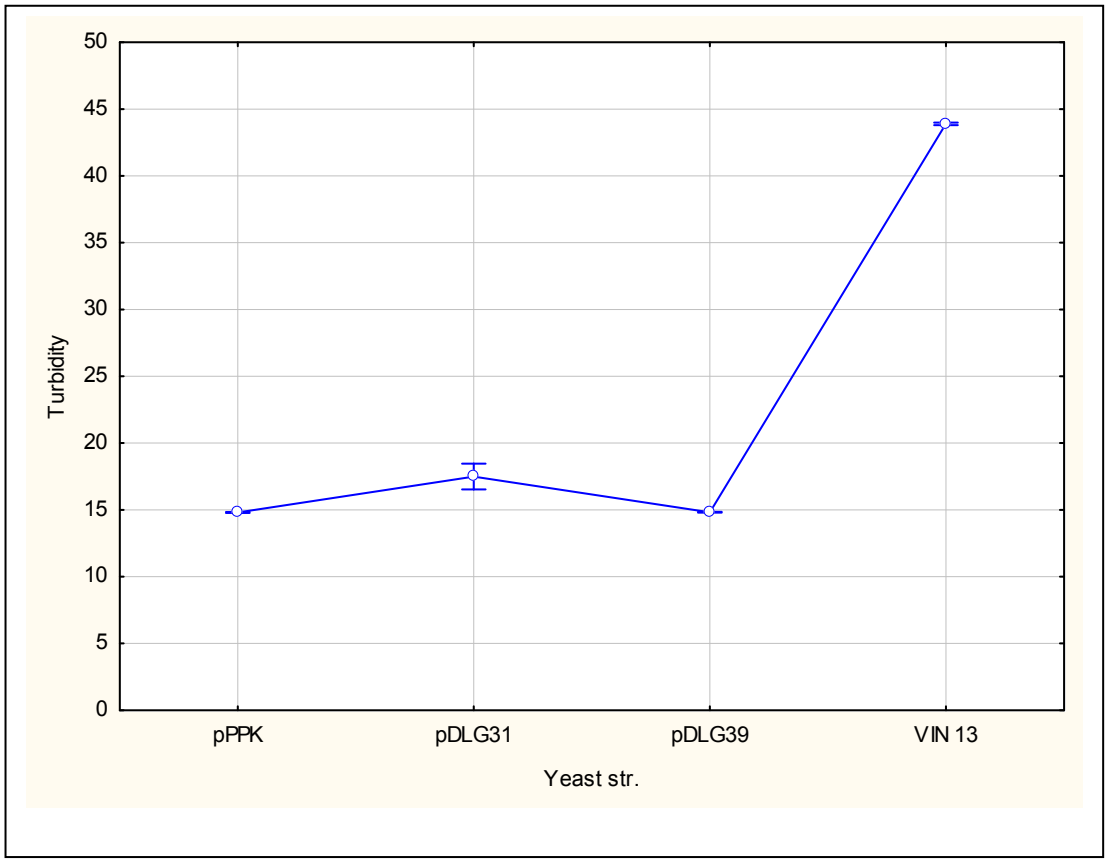

Figure 5.3 (a): The turbidity results of wines from the Bogoya cultivar fermented with recombinant yeast.

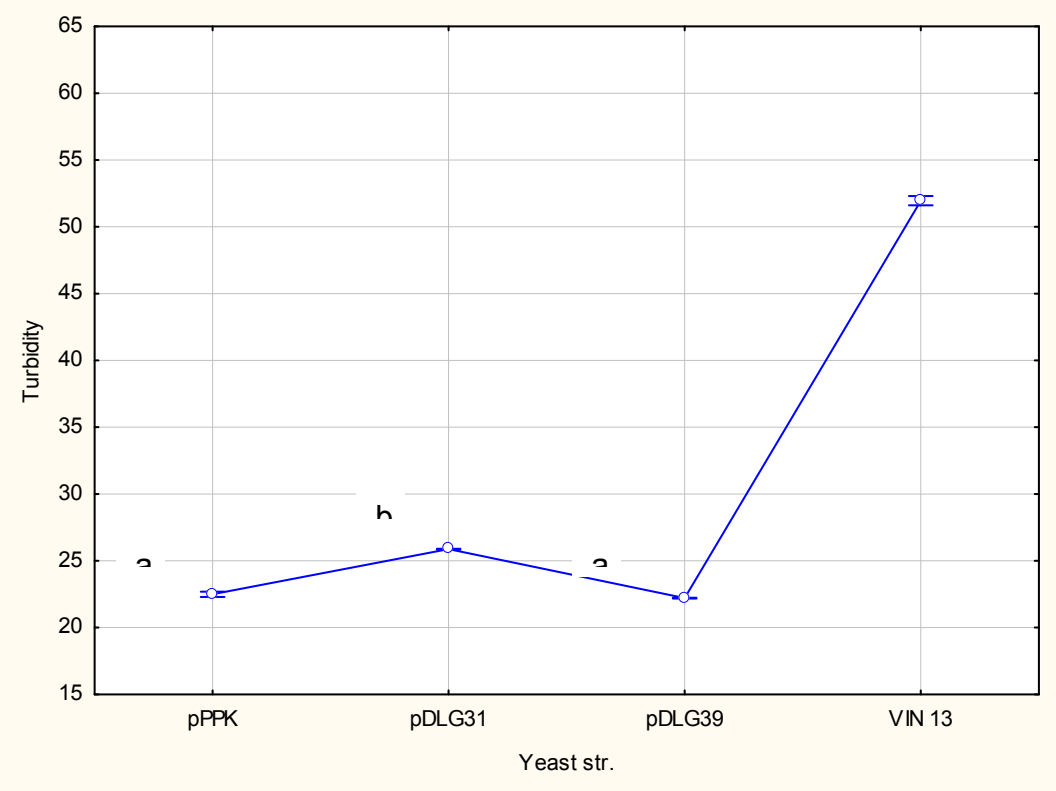

Figure 5.3 (b): The turbidity results of wines from the Kayinja cultivar fermented with recombinant yeast.

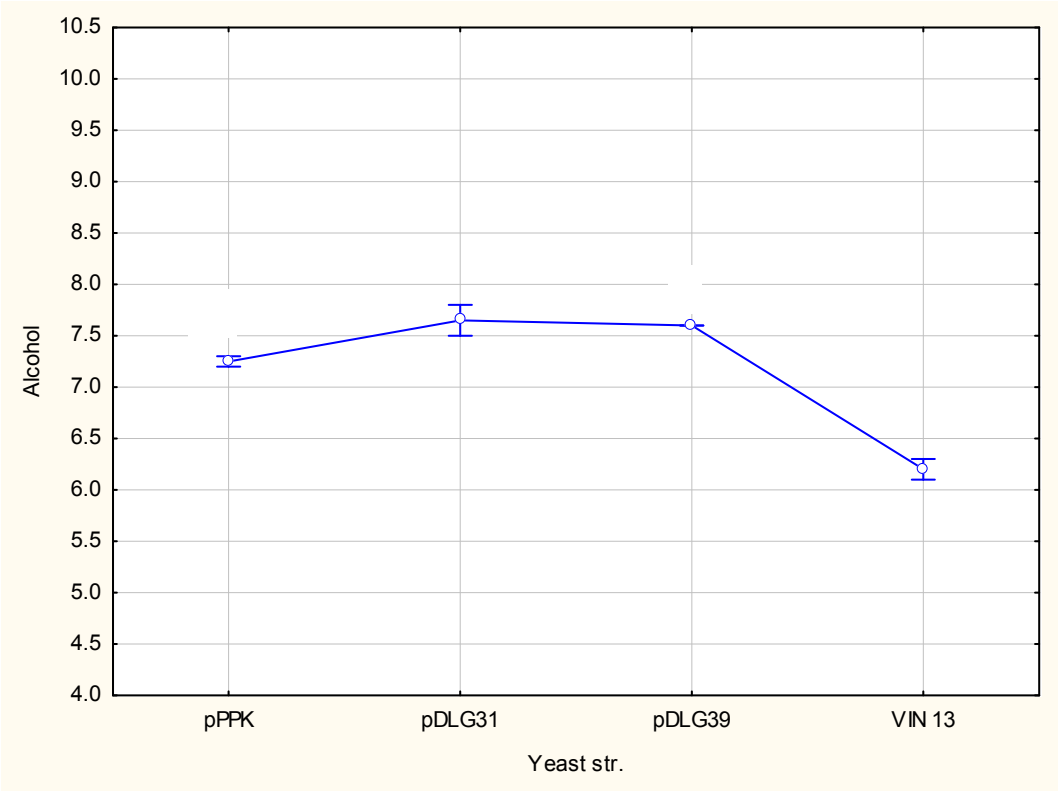

Figure 5.4 (a): Alcohol levels (%v/v) obtained in Bogoya wines.

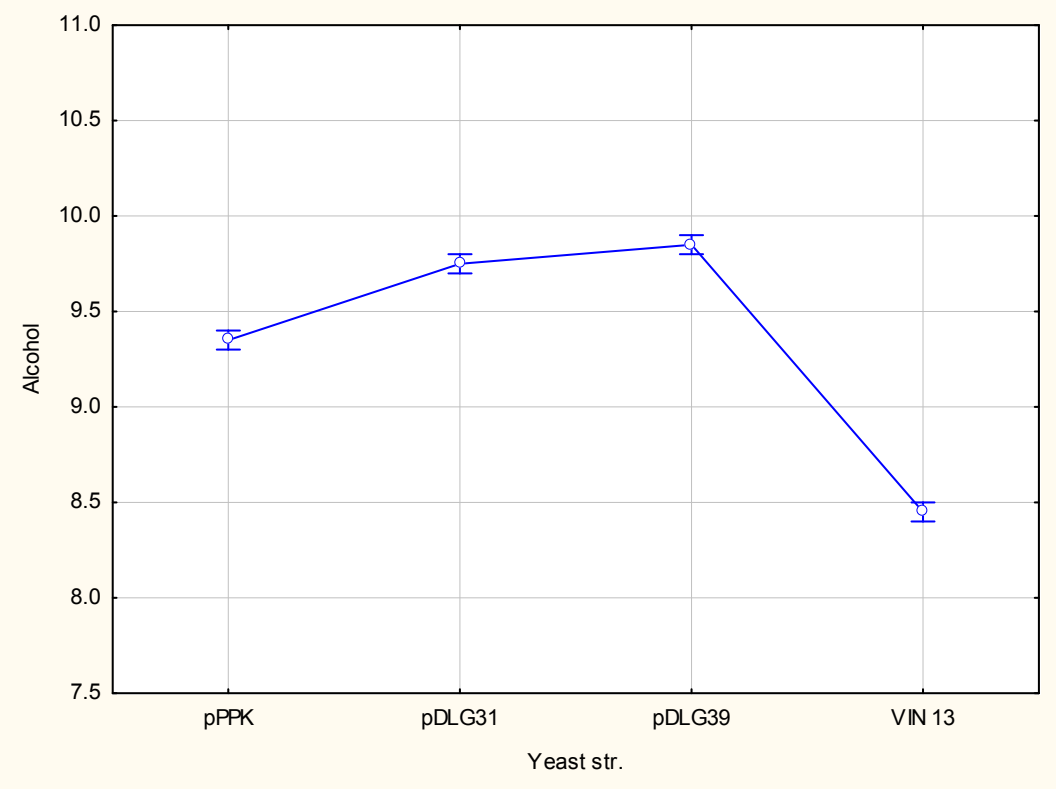

Figure 5.4 (b): Alcohol levels (%v/v) obtained in Kayinja wines.

In this study, stable wines were produced with the use of recombinant yeast strains. It seems that using enzyme producing recombinant yeast was more effective compared to using the commercial enzymes on its own. In some cases the volume of wines obtained was more compared to the volume of juice extracted in Chapter 3. This may due to longer reaction time during the fermentation compared to the 24 hr incubation time with the commercial enzymes.

Kreuzer and Massey (2001), stated that the traits that are being introduced through genetic engineering are similar to those produced through selective breeding, and so the overarching concern about the safety of genetically engineered foods compared to our current foods is misplaced. The GM yeasts used in this study have been developed and used following the appropriate scientific protocols. As far as we know this is the first report of the use of recombinant yeast for the production of banana wine and may generate some information for further research and processing techniques in the field of biotechnology for production of novel foods.

5.4 CONCLUSION

The wines produced with the recombinant yeast definitively displayed some positive aspects. More wine was obtained compared to the control, more alcohol was obtained and the wine was much clearer than the control wine. The recombinant yeast strains may serve as alternative to commercial enzymes in processing the banana wines. These recombinant yeast starter cultures could play an important role in rural areas, where it would be extremely difficult to store the various commercial enzymes under the correct conditions. They would also help to produce a less expensive, stable product from “waste” bananas. A further important analysis that still needs to be determined is whether the aromas and taste of the GM wine are similar or totally different from the non-GM produced banana wines.

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CHAPTER 6
GENERAL DISCUSSION AND RECOMMANDATIONS

6.1 DISCUSSION

Bananas are grown widely in the tropical and subtropical world and, in terms of production, are the fourth most important crop after rice, maize and wheat. Bananas are utilised as food in various forms, i.e. cooked, fried, baked, roasted and raw dessert, and as beverages in the form of juice and beer. Different researchers (Jackson and Badrie, 2002; Akingbala *et al*., 1994; Gous *et al*., 1987; Mabesa *et al*., 1989; Kyamuhangire and Pehrson, 1998; Gensi *et al*., 2000; Koffi *et al*., 1991; Shahadan and Abdullah, 1995; Kyamuhangire *et al*., 2002; Sims and Bates, 1994; Sims *et al*., 1995; Viquez *et al*., 1981; Davies, 1993; Munyanganizi-Bikoro, 1975; Joshi *et al*., 2000) have studied juices and wines from different banana cultivars and some of them have come up with acceptable banana wines (Kundu *et al*., 1976; Akingbala *et al*., 1992; Jackson and Badrie, 2002).

However, banana beverages have remained of low quality in many banana-producing countries, mainly due to a lack of added value. For this reason, these banana beverages have the least competitive advantage with other tropical fruit juices and wines and are least exported nationally and regionally. Therefore, the need to further investigate banana juice and wine through research with the aim of improving the quality of the product cannot be overemphasised. Commercial enzyme application in banana beverage processing procedures seems to be one of the cornerstone areas to be exploited further at both laboratory and industrial level in order to increase production and improve the quality of the banana juice and wine. Mixtures of pectinase, cellulase and hemicellase enzymes were reported (Sims and Bates, 1994) effective in reducing viscosity and improving the filterability of puree from both green and ripe bananas. Such enzyme mixtures should be fully exploited for production of banana based beverages of sustainable economic benefits.

In this study, it has been elucidated that enzymes play a very important role in juice extraction and clarification, without significant differences in the other major parameters required for qualitative banana beverages of the three cultivars studied. Higher juice yields were obtained (see Tables 3.2 and 3.3). General turbidity of the

banana juice and wine was reduced significantly by enzymatic action (see Tables 3.5 and 4.2).

Banana fruit as raw material for processing into juice and wine can be described as a very sensitive and delicate. It has been experienced practically that certain parameters deteriorate quickly, especially on exposure to atmospheric oxygen. The defects include acidification, browning and off-flavours in the beverages. Whereas a little oxygen is necessary at the beginning of fermentation for the yeasts to increase their biomass, any further oxygen ingress in the fermenter or the bottled banana wine leads to the conversion of alcohol to acetaldehyde and acetic acid (vinegar flavour) by acetic acid bacteria (AAB). In their discussion of AAB and wine, Bartowsky and Henschke (2004) reported that all is well until oxygen enters the scene. This undesirable situation was encountered once in this study. In the worst circumstances, visible spoilage characteristics of a distinctive deposit, described as a circular ring in bottled wine by Bartowsky and Henschke (2004), are seen as a white floating layer. This massive growth of AAB is insoluble in water and some organic solvents. The bacterial growth responsible for the wine spoilage occupies part of the headspace or is present below the closure when there is oxygen ingress and causes an unpleasant smell in the wine. Previous studies have shown that *Acetobacter* spp. are able to survive for quite extended periods of time, even in anaerobic conditions and can proliferate when exposed to any small concentrations of oxygen (Joyeux *et al*., 1984; Millet and Lonaud-Funel, 1999).This means that, during the fermentation of banana juice, oxygen must be avoided because even the little that may enter during sampling or transfer from one vessel to another may be enough to cause spoilage in the sensitive banana wine.

In brief, the measures that were used to minimise oxygen entry into banana wine and stop wine spoilage by AAB included the use of sulphur dioxide (SO_2), the creation of tight anaerobic conditions, and drawing samples for analysis under pressure in a tightly sealed fermenter. They also involved the production of large batches of must to leave a small fermenter space and to reduce the surface area to volume ratio in order to manage the biomass better and decrease oxidation. Further measures aimed at increasing the level of ethanol, which is inhibitive to bacteria at 12% (v/v) ethanol, storing the bottled wines in a horizontal position to avoid oxygen ingress, and sterile filtration. However, sterile filtration has negative effects on the quality of

wine. Decreasing the pH to inhibitory levels (pH 3.3 or lower for LAB), as reported by Du Toit and Lambrechts (2002) and Du Toit and Pretorius (2002) will help, although growth of AAB was much greater in South African red wines due to higher pH.

Regarding TSS and pH of juice to be used for winemaking, some adjustments may be made if required. In case of low TSS in the juice, chaptalization (addition of sugar) would be done to raise the brix of the juice. Juices and musts that fail to possess the desired acidity and pH may be adjusted before fermentation as described by Jackson (2000), Iland *et al*. (2000) and Boulton *et al*. (1996). The volatile compounds of grape wine and banana wines (see Appendix 6 and Table 4.6) have been compared. It was established that some volatile compounds are present in banana wine in higher concentrations than in grape wine. It was also noted that some volatile compounds found in grape wine are not found in banana wine and vice versa.

Results from this study have shown that the commercial enzyme preparations that were used play a big role in influencing juice yield in banana processing and, on the basis of a previous report (Voragen *et al.*, 1986) that technical enzyme preparations are used widely in the fruit-processing industry to facilitate juice release and increase yields, bananas should be among the fruits to be processed using commercial enzyme preparations for juice production. Juice and wine clarification in terms of turbidity reduction was also greatly improved. This is achieved mainly due to the degradation of starch and pectin from the fruit and a reduction in viscosity (Koffi *et al*., 1991).

Usually, well-flavoured beer with a bitter taste, brown-golden colour and low alcohol content (2 to 5% v/v) is produced from diluted juices(for a details on banana beer processing, see Kyamuhangire and Pehrson,1999; Gensi *et al*., 2000). Strong banana beer with an alcohol content of 11 - 15% is produced from undiluted juice (Davies, 1993). This banana beer has an average shelf-life of about five days.

The alcohol percentages obtained in this study ranged between 5.6 and 14.6% v/v, depending on the banana cultivar and the degrees brix of the banana fruit at harvest. The alcohol content obtained in banana wine is in the range of that that was achieved in banana beer and in comparison the banana wine has a longer and more stable shelf life than the banana beer.

This alcohol content range is not far from table wine alcohol levels and is close to the findings of previous researchers (Jackson and Badrie, 2002; Akingbala *et al*., 1994). The alcoholic content of the banana wine was highest with Kayinja. This is related to the high sugar content (part of TSS) of this cultivar. The enzymes that cause better digestion and degradation of starch and pectin in the banana pulp caused longer fermentation periods than the controls and enhanced higher yields from the treated pulps.

The wine made with Kayinja bananas therefore could be produced at pilot-plant scale to test it in the market in the near future in countries like Uganda, where bananas are produced in bulk and there is no alternative source for winemaking.

The treatment of the pulp with commercial enzymes (Rapidase CB, Rapidase TF, Rapidase X-press and OE-Lallzyme) produced juice and wine with a sensory profile that was not significantly different ($p>0.05$) from the untreated control. The overall acceptability of juice from the three banana cultivars scored between 4.9 and 7.8 on the nine-point hedonic scale. Juice sensory evaluation showed that the most preferred banana juice was that extracted from Kayinja (*Musa*, cultivar ABB), the cultivar in which the highest sugar content had been obtained. This cultivar therefore had the sweetest taste, which seems to have been a major attribute in determining overall acceptability by the assessors. The acidity level was also judged least in Kayinja juices and could have been another factor contributing to greater preference for its juice regarding overall acceptability. However, the overall acceptance of the juices scored highest in the control juices, which may not be a mere coincidence, as it has previously been speculated by other researchers (Mabesa *et al*., 1989; Kyamuhangire *et al*., 2002) that enzymes as additive aids in processing may alter certain attributes and render the banana juice less natural. Previous research (Kyamuhangire *et al*., 2002) has reported that the overall acceptability of the enzyme-extracted banana juice was affected by its slightly astringent taste. In this study, the more than 50% overall acceptability shows that there is potential for consumption.

The sensory evaluation of the banana wine also had an overall acceptability score of over 50%, with the lowest score of 4.6 being allocated to the Mbidde pulp that had been treated with Rapidase TF. The highest score was 7.5 on the nine-point scale for the control of wine from Kayinja.

The results of the tasting by the panellists are in agreement with previous research by Akingbala *et al*. (1994), who reported that acceptable table wines were prepared from the juice of over-ripe banana fruit.

By the end of this study, it was clear that enzymatic juice extraction and clarification of both banana juice and wine are rewarding, both quantitatively and qualitatively. It was also determined that overripe bananas that may be available in bulk should not be wasted, as they can be processed with minor problems to produce safe and quality beverage products. This study has confirmed that the banana cultivar (*Musa*, AAA genotype), which has always been regarded as a dessert fruit, can be a source of juice and wine with acceptable characteristics in its overripe state.

6.2 RECOMMENDATIONS

Following the application of commercial enzymes for the processing of banana juice and wine in this study, the following are recommended for future exploitation:

(a) Extraction of juice and wine from over-ripe Bogoya (*Musa*, AAA genotype).
(b) Production of banana juice and wine by small-scale industries using enzymes.
(c) Using recombinant wine yeast strains to produce banana wine as a novel product.

6.3 SUGGESTIONS FOR FUTURE WORK

1. In this work, there was no blending of banana juice and wine with other fruit cultivars or different fruit juices. This may be done under enzymatic processing in future work to monitor if there may be better quality properties in banana juice and wine.
2. Amelioration may be one of the means to improve quality of banana wine further. This may include chaptalization (addition of sugar) to must to improve the alcohol content in wine, if so desired by the wine consumers.
3. Future work investigations may necessitate further studies to find out the most appropriate enzyme mixtures for viscosity reduction, the temperature and

enzyme-mash contact time for maximum juice yields in various banana cultivars.

4. More research may be designed to investigate and establish when (after how long) the protease enzymes would actually cease to effect any further haze stabilization (clarification) in banana wine.

6.4 REFERENCES

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APPENDICES

Appendix 1: List of Acronyms

AAA	acuminata triploid genome group
AAA-EA	acuminata triploid, East African group
AAB	Acetic acid bacteria
ABB	acuminata, balbisiana diploid genome group
Acceptab.	Acceptability
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
^{0}B	Degrees brix
CAC	Codex Alimentarius Commission
CBD	Convention of Biological Diversity
CFU	Colony formed units
$_{C}$P	Centipoises
DAP	Diammonium phosphate
DAHP	Deoxy-D-arabinohetulosonate-7-phosphate
DNA	deoxyribonucleic acid
FAO	Food and Agricultural Organisation
FAN	Free available nitrogen
g-force	Gravitational force
GC	Gas chromatography
GM	Genetically modified.
GMOs	Genetically modified organisms
GMP	Good manufacturing practices
GRAS	generally recognised as safe
HACCP	Hazard analysis critical control point
HPLC	High power liquid chromatography
Hr	Hour
hL	Hectolitre
g/L	Grams per litre
IITA	International Institute for Tropical Agriculture
INIBAP	International Network for Improvement of Banana and Plantain
ISO	International Standards Organization
IWBT	Institute of Wine Biotechnology
LAB	Lactic acid bacteria
LSD	Least significant difference
MDGs	Millennium development goals
NTU	Nephelometric turbidity units
NBS	National Bureau of Standards
OE-Lallz.	OE-Lallzyme
PE	Pectin esterase
PG	Polygalacturonase
PME	Pectin methyl esterase
ppm	parts per million
PPO	Polyphenoloxidase
Rap.CB	Rapidase CB
Rap.TF	Rapidase TF
Rap.X-press	Rapidase-X-press
SA	South Africa
spp	Species

SO_2	Sulphur dioxide
TA	Titratable acid
TBT	Technical Barriers of Trade
TSS	Total soluble solids
UCDA	Uganda coffee development authority
UN	United Nations
USA	United States of America
U Shs	Uganda shillings
VA	Volatile acidity
WHO	World Health Organization
WTO	World Trade Organisation
YPD	Yeast peptone dextrose

Appendix 2: Banana Production in East Africa and South Africa.

Figure A2 (a) shows the East African growing areas with the dark shaded areas and Figure A2 (b) shows South African banana production localities. Appendix 3 shows the world banana producing countries.

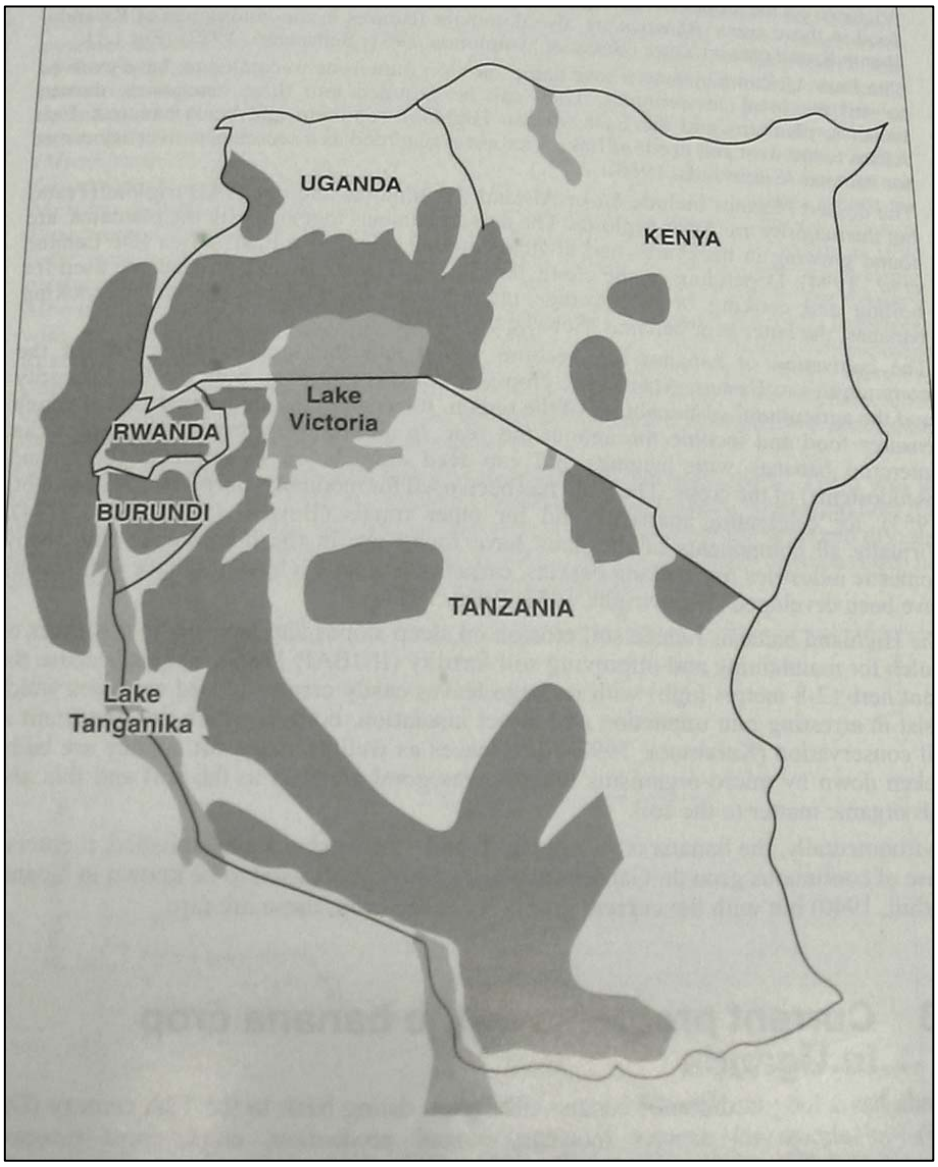

Figure A2 (a) : East African banana producing areas.

Source: Karamura , D.A. 1998. Numerical taxonomical studies of the East Africa highland bananas (*Musa*-AAA-East African) in Uganda. CARPAC-France. PhD dissertation, University of Reading, UK.

Figure: A2 (b) : South African Banana growing areas

Source: ARC-Institute for Tropical & Subtropical Crops (ARC-ITSC) .

Appendix 3: World Banana Production

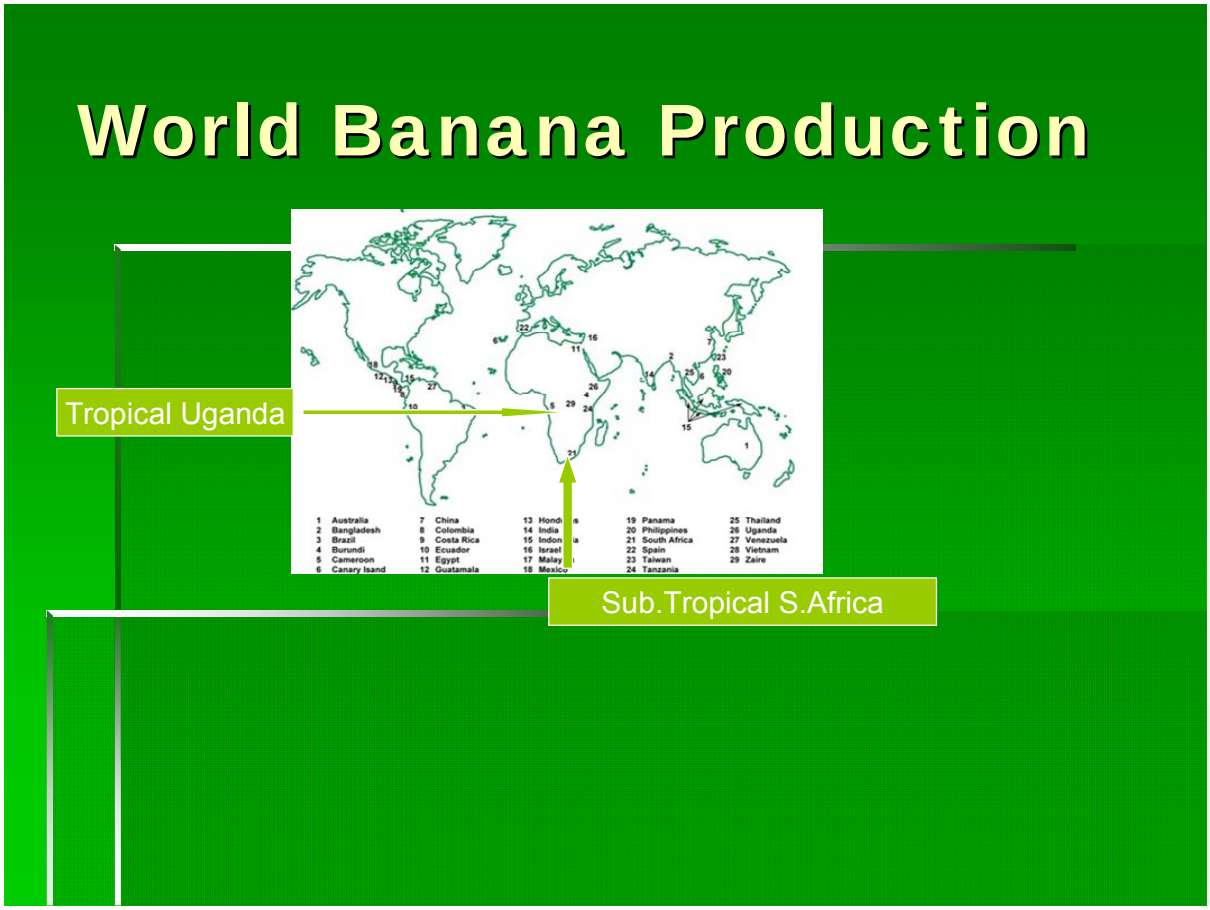

Figure 3: World banana producing countries

Source: INFO COMM, 2005. Market information in the commodities area. Banana Production http://r0.unctad.org/infocomm/anglais/banana/ characteristics. htm 19/12/2005

Appendix 4: Banana wine sensory attributes observed after ANOVA test

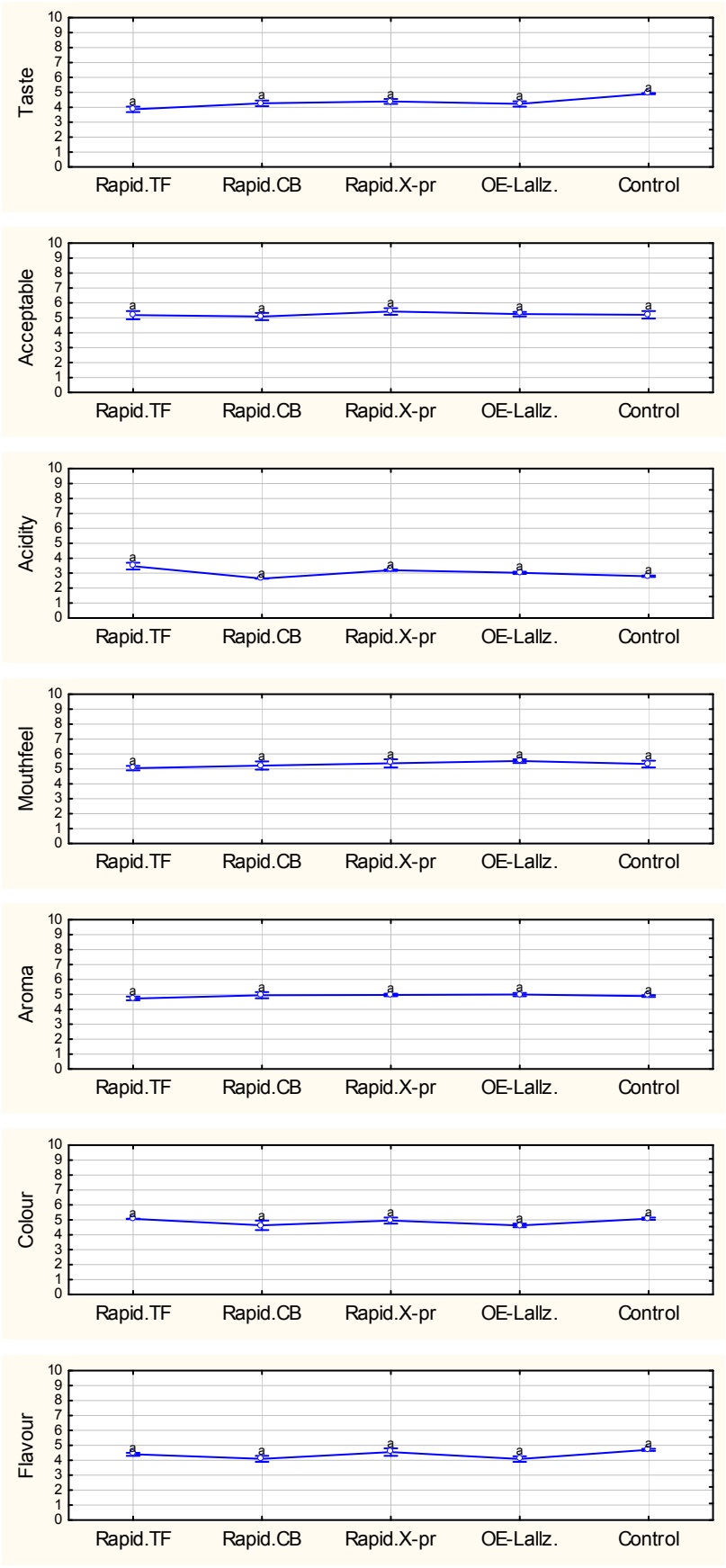

Figure 4 (a): Bogoya (*Musa,* AAA genotype) banana wine attribute levels

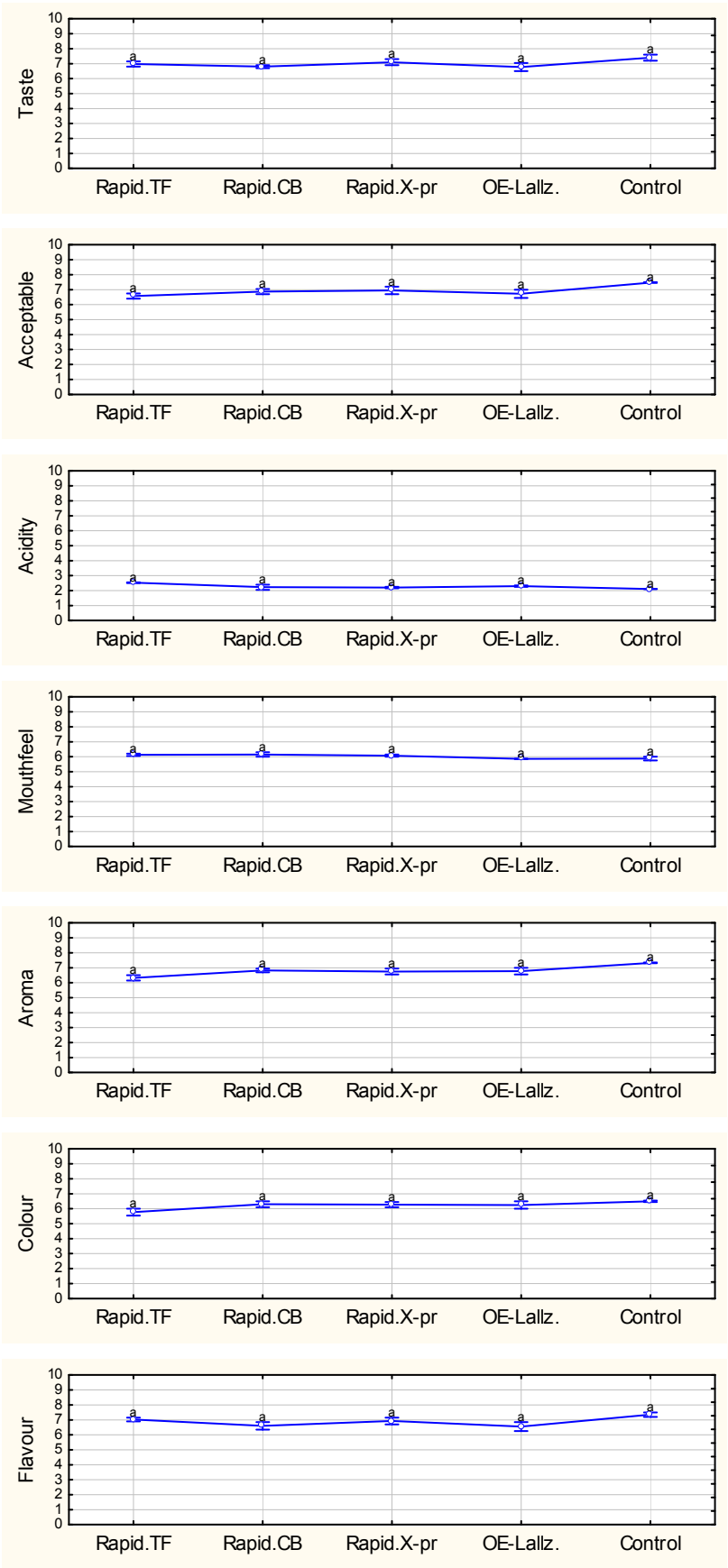

Figure 4 (b) : Kayinja (Musa, ABB genotype) banana wine attribute levels

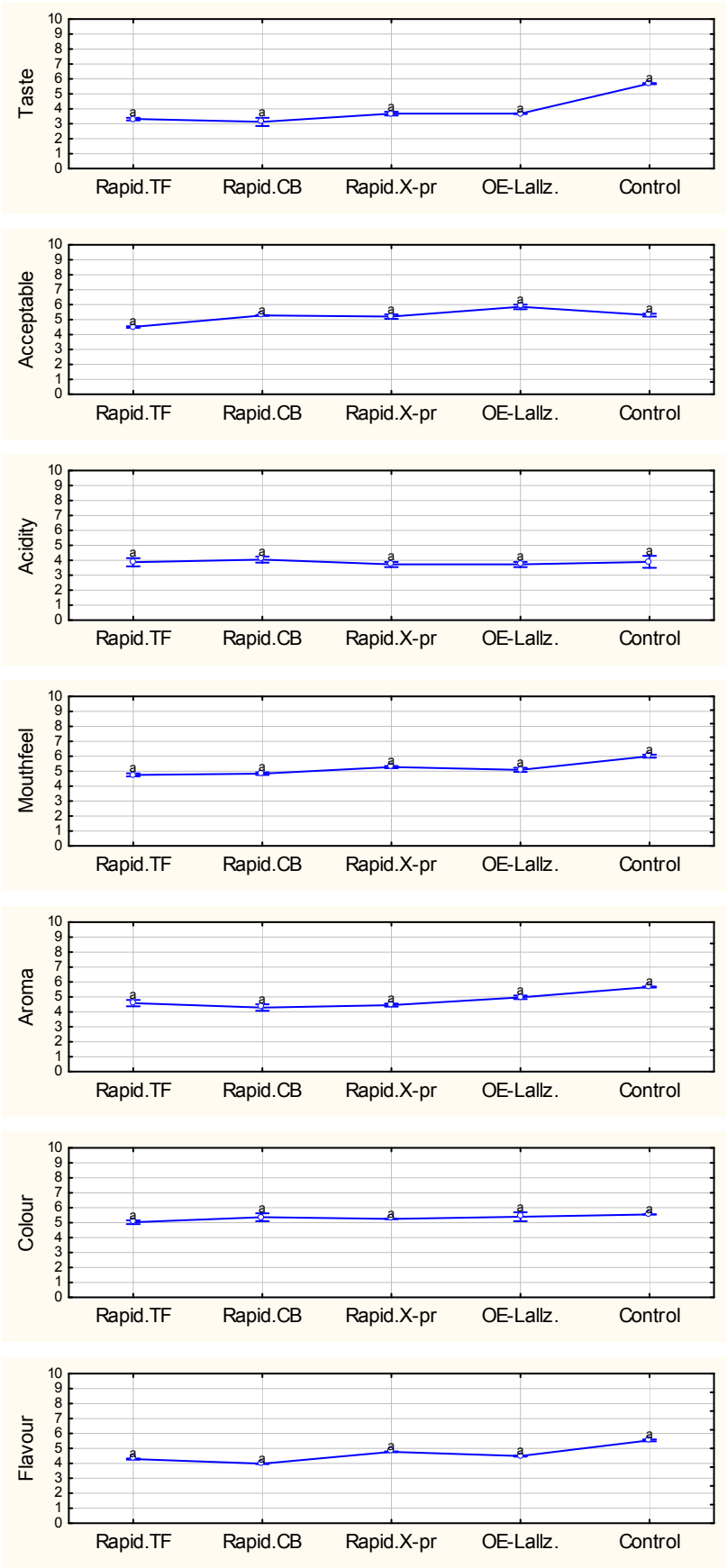

Figure 4 (c) : Mbidde (*Musa*, AAA-EA genotype) banana wine attribute levels

Appendix 5 (a): Composition of wine excluding phenolic acids and polyphenols

Component	Conentration(g/100 ml)
Water	80-90
Carbohydrates[a]	
Glucose	0.05-0.1
Fructose	0.05-0.1
Pentose	0.08-0.2
Arabinose,rhamnose,xylose[b]	
Pectin	Trace
Inositol	0.03-0.05
Fucose	0.0005
Alcohols	
Ethyl	8.0-15.0
Other	
Methyl,higher,2,3-butylene glycol,acetoin[b]	0.3-0.19
Glycerol	0.30-1.40
Aldehyde	0.001-0.050
Organic acids	0.3-1.10
Tartaric,lactic,succinic,acetic,*p*-hydroxy-glutaric galacturonic,malic,citric,fumaric,oxalix α -ketoglutaric,aconic,citra-malic,malonic, pyrorocemic,pantothenic[b]	
Nitrogenous compounds	
Amino,ammonia,amide,protein humin[b]	0.01-0.09
Mineral compounds	0.15-0.40
Potassium,magnesium,carbon dioxide,phosphate Sulfate,calcium,chloride,silicic acid, fluoride, aluminium,manganese,sodium,iron,boron,iodine, copper, rubidium,oxygen[b]	
[a]Concentration is dependent on style of wine,that is dry or sweet [b]Indjvdual compounds are ranked in decreasing concentrations	

Source: German and Walzem (2000).The Health Benefits of Wine, Annu. Rev. Nutr. 20.p.569.

Appendix 5 (b): Phenolic and polyphenol components of red and white wines[a]

Component	Concentration (mg/L)	
	Red wine	White wine
Nonflavonoids	240-250	160-260
Hydroxybenzoic acids	0-260	0-100
p-hydroxybenzoic acid	20	_
Gallic acid	116(26-320)	1.4
Total gallates	40(30-59)	7(6.8,7.0)
Salicylic acid	_	_
Syningic acid	5(4.2-5.9)	_
Protocatechuric acid	88	_
Hydroxycinnamic acids	162(62-334)	130-154
cis/trans-Coutaric	20(16-24)	1.8
cis/trans-Caftaric	25(11-47)	5(3,7)
Caffeic acid[b]	8.5(3-18)	2.8
Coumaric acid[b]	12.6(7.5-22)	1.5(1-2)
Ferulic acid[b]	19	_
Stilbenes	12.3(4-19)	1.8(0.04-3.5)
trans-Resveratrol	1.0(0.1-2.3)	0.22(0.003-2.0)
Flavonoids	750-1060	25-30
Flavonols	98(10-203)	Trace
Quercetin	18.8(5-53)	0
Myricetin	16.2(2-45)	0
Kamempferol	18	0
Rutin	6.8(0.5-10.8)	0
Flavanols	168(48-440)	15-30
Catechin	89(27-191)	17.3(3-35)
Epicatechin	57.3(21.4-128)	13.6(2,18.9,21)
Procyanidins	171(29-333)	7.1(5-10)
Anthocyanins	281(20-500)	0
Delphinidin 3-monoglucoside[c,d]	22	0
Cyanidin 3-monoglucoside[c,d]	20(2.8, 38)	0
Petunidin 3-monoglucoside[c,d]	18	0
Peonidin 3-monoglucoside[c,d]	32	0
Malvidin 3-monoglucoside[c,d]	93(24-170)	1
Total phenolic acids and polyphenols	1200(900-2500)	200(190-290)

[a]Tubular values are reported as milligrams per liter withvalues drawn from(45,54,56,96,103,107,125,154,160,171-173,185).Not all authors reported all compounds or classes ofcompounds.Mean values were calculated from all avilable values.The range of contributing values is shown in parentheses,whereas range values separated by a comma are individual values contributing to an average.Values without a range are the sole value found .In some instances,only ranges were reported and are shown without a mean value.Dashes indicate no value found in literature.

[b]Also present as tartrate esters.

[c]Also present as a diester with acetate.

[d]Also present as a diester with p-coumarate.

Source: German and Walzem (2000).The Health Benefits of Wine Annu. Rev. Nutr. 20. p. 570.

Appendix 6: Examples of volatile compounds found in several wines analysed by Gas Chromatography

Compounds (mg/L)	WINE1	WINE2	WINE3	WINE4
Methanol (ml/L)	0.026	0.030	0.028	0.032
1-Propanol	11.0	18.5	20.0	18.0
2-Methyl-1-popanol (isobutanol)	10.0	13.0	25.0	34.0
1-Butanol	2.5	2.5	0.5	0.5
2-Methyl-1-butanol	20.0	22.0	16.5	17.0
3-Methy-1-butanol	114.0	120.0	105.0	111.0
Total higher alcohols	157.5	176.0	167.0	180.5
Acetaldehyde	52.0	56.0	37.0	31.0
Ethyl acetate	31.0	33.5	25.0	25.0
isobutyl acetate	0.018	0.020	0.014	0.018
Isoamyl acetate	2.4	2.2	0.85	0.92
n-Hexyl acetate	0.13	0.15	0.16	0.17
ß-phenyl-ethyl acetate	4.0	3.5	0.12	0.13
Ethyl butyrate	0.13	0.13	0.10	0.11
Ethyl caproate (C6)	0.91	0.71	0.86	0.83
Ethyl caprylate (C8)	1.4	1.0	0.60	0.66
Ethyl caprate (C10)	0.28	0.24	0.10	0.11
Ethyl 9-decanoate	0.002	0.004	0.005	0.006
Ethyl pyruvate	0.23	0.12	0.33	0.16
Ethyl lactate	3.8	4.0	4.8	4.2
Diethyl succinate	0.41	0.81	0.12	0.11
Ethyl succinate	5.9	9.5	4.2	2.3
Acetoin	0.35	0.30	0.45	0.29
Diethyl malate	0.58	0.86	1.4	1.0
y-Butyric lactone	3.3	4.6	3.5	3.3
4-hydroxy ethyl butyrate	1.7	2.6	1.6	2.4
Pantolactone	0.11	0.12	0.14	0.10
4-Carboethoxy γ-Butyrolactone	0.22	0.29	0.24	0.25
2-ethyl hydroxyglutarate	0.04	0.12	0.08	0.06
3-Methylpentanol	0.045	0.053	0.016	0015.
4-methylpentanol	0.004	0.005	0.005	0.006
Hexanol	0.70	0.92	1.5	1.4
Trans 3-hexanol	0.061	0.061	0.042	0.072
Cis 3-hexanol	0.046	0.060	0.056	0.065
Benzyl alcohol	0.059	0.021	0.056	0.035
ß-phenethyl alcohol	15.0	17.0	18.5	16.8
3-Ethoxy-1-propanol	0.63	0.95	1.1	0.68
3.thiomethyl-1-propanol	1.2	1.4	1.0	0.97
3-thioethyl-1-propanol	0.043	0.044	0.017	0.012
1,3-Propanediol mono acetate	0.82	1.3	1.9	1.4
1,4-butanediol acetate	0.28	0.36	0.50	0.41
Ho-diol (1)	0.023	0.031	0.044	0.038
Isobutyric acid (IC4)	0.5	0.7	0.5	0.5
Butyric acid (C4)	1.3	1.4	0.73	0.64
Isovaleric acids (C5)	0.8	0.9	0.5	0.5
Caproic acid (C6)	4.9	4.1	5.5	5.5
Caprylic acid (C8)	10.8	8.4	8.7	8.7
Capric acid (C10)	2.9	2.5	2.2	2.4
9-Decanoic acid	0.058	0.075	0.016	0.006
N-(3 methylbutyl) acetamide	2.5	2.0	1.4	2.8

Source: Delfini and Formica (2001). Wine microbiology, science and technology.p.257.