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Fermentation Capacity of Yeasts Using Mango (*Mangifera indica* Linn.) as Substrate

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Abstract

The goal of this study was to address the problem of large post harvest losses of mangoes by employing yeast fermentation technology to produce a more stable, value-added product in this case fruit wine. The design of the study involved determination of the fermentative capabilities of four commercial yeast types on musts obtained from an improved (Keitt) mango cultivar that is popularly cultivated in some parts of Ghana for export. The characteristics of the mango musts that were monitored included total soluble solids (TSS), pH and total acidity (TA), microbial populations (aerophilic mesophiles, yeasts and Acetic acid Bacteria), alcohol content and colour over the course of the fermentations. Descriptive and hedonic sensory evaluation was carried out on the ferments obtained from all treatments. Results showed that two of the yeast types namely; Red Star Pasteur and Red Star Montrachet displayed superior fermentation characteristics and produced mango wines that were acceptable by both descriptive and affective sensory panels.

Keywords: mango, yeast, fermentation, alcohol, wine, must, soluble solids, acidity.

1. Introduction

Mangifera indica (Mango) is one of the most important tropical fruits and six varieties, which include Kent, Keitt, Haden, Tommy Atkins, Palmer and Zill are commercially cultivated in Ghana. Until recently mangoes were either picked from the wild (FAO, 2009), or their commercial cultivation was done by small-holder farmers who sold them fresh in local markets with little processing or value addition. Increased global interest in the fruit (Tharanathan *et al.*, 2006; Evans, 2008) has transformed them into international items of commerce with huge export earning potential. In Ghana, a great deal of commercial production is targeted at the growing European fresh fruit market (Ghana Export Promotions Council, 2005; Sauco, 2004). However, trade restrictions, together with high rejection rates due largely to the farmers' inability to meet specifications, make it difficult for export of the fruits and consequently cause the produce to end up in the local market. This sets up a yearly cycle of bumper harvests, with little capability for preservation or value addition, leading to huge post harvest losses. This situation is further exacerbated by poorly established practices for handling, transportation, storage, and ripening of the fruit (Bello-Pérez *et al.,* 2007). Although there is very little empirical data in Ghana to describe the post-harvest loss situation of the fruit, it is generally believed to be between 10 - 30% or higher. Apart from the need to improve the efficiency of post harvest handling and preservation, it is also necessary that value addition steps be taken for the fruit's management in order to realize full benefits from them. Agro-processing of mangoes into products of high value and long-shelf life will help mitigate the huge post-harvest losses and diversify utilization of the fruit (Gitonga *et al.,* 2010).

The mango fruit, which typically has high fermentable sugar composition when mature and ripe (Tharanathan *et al.*,, 2006), could be exploited as a substrate for alcoholic fermentation. A careful choice of fermenting yeasts under enabling conditions could turn mango pulp into a more stable and acceptable product, such as fruit wine, thus transforming an otherwise perishable produce to a high value and stable product. The objective of the study was to evaluate the fermentative capacities of four yeast strains on mango pulp as substrate for alcoholic (wine) fermentation.

2. Materials and Methods

2.1 Sample Collection and preparation

Ripe matured fruits of mango (Keitt, variety) were obtained from mango growers along the Dodowa-Somanya road in the Greater Accra Region in Ghana. The fruits were washed, rinsed in distilled water and air-dried. They

were sanitized by submerging in potassium metabisulphite solution (350ppm) for one (1) hour and then air-dried. The fruits were weighed and manually peeled. They were then macerated into pulp at high speed in a Warring blender. The specific gravity (SG) and percent °Brix of the pulp were determined. Potassium metabisulfite, was added to the macerated pulp at a concentration of 100 mg/l (Duarte, 2010a) and the pulp was then left to stand overnight. Sucrose solution was added to the fruit pulp to adjust the total soluble solids content to 22 °Brix. Pectinase (BSG HandCraft, USA) at a concentration of 4g/l was added to the must, stirred and then left overnight at 22 ± 2°C for the pectinase to hydrolyze the pectin and enhance the juice extraction. Other additives such as yeast nutrient (Di-ammonium Phosphate and Ammonium Phosphate), tannin powder were added to the must. The pH of the must was adjusted to 3.9 by adding a mixture of organic acids where necessary.

2.2 Alcoholic Fermentations

Four commercial active dry yeast cultures used in the study included Red Star® Montrachet, Red Star® Pasteur Champagne, Lalvin D47 and Anchor Brewer's yeast. Two of the yeast strains: Red Star Montrachet (RSM) and Red Star Pasteur (RSP) Champagne yeast (produced by Fermentis, a Division of S.I. Lesaffre group – USA) and the Lalvin® yeast culture (produced by Lallemand – Canada) were procured from a commercial wine supply shop in Virginia, USA. Anchor® brewer's yeast (produced by Lallemand - Canada) was procured from the GAME shopping mall in Accra, Ghana. Fermentation was carried out using 250ml of chaptalised mango must (22 °Brix) as fermentative substrate. The must was inoculated by the addition of 0.4g/l of the rehydrated active dry yeast culture. All vinifications were carried out in 300ml flasks at room temperature 27 ± 3°C. Fermentation indices monitored included °Brix, pH, total acidity, alcohol content, colour, and change in microbial populations. The fermentations were considered complete when the °Brix reached a stable level and there was no recorded change after 48 hours. All assays were in duplicates.

2.3 Racking and ageing

Racking was done when total soluble sugars (TSS) reached 4-5 ºBrix without a further drop occurring for the next 48 hours by siphoning the fermented (supernatant) liquor into a clean vessel. Three more rackings were done at 15 days interval to remove any sediment deposited in the beverage. After three months of bulk ageing, the wine was transferred into bottles (filled with very little head space) and stored under refrigeration (10-15ºC) for 3 months. The process flow chart is shown in Figure 1.

2.4 Physico-Chemical Analyses

Percent Total Soluble Solids or ^{o}Brix of the must samples was determined using a refractometer (AOAC, 1990). The pH of the samples was determined using a digital pH/conductivity meter equipped with a temperature sensor (OAKTON deluxe waterproof pH/ Conductivity meter kit, model No. 35630-62). Determination of total acidity was done as per the official methods, (AOAC, 1990) using phenolphthalein as the indicator for the titration endpoint against 0.1N NaOH. Total acidity was calculated as percent citric acid while volatile acidity was calculated as percent acetic acid (Saritha *et al*., 2010). Vitamin C was determined using the 2,6-dichlorophenol-Indophenol dye method (AOAC, 1990). Alcohol content (by volume) of the fermented samples was determined using a pycnometer according to the protocol outlined in AOAC (1990).

2.5 Product Colour (CIELAB) determination

Colour measurements (L*, a*, b*) of the fresh must and fermenting must were carried out using a Hunter Lab colorimeter (Chroma-Meter CR 200, Minolta, Germany) calibrated against both white and black colour tiles before each use (Hung, 1990; OIV, 2011).

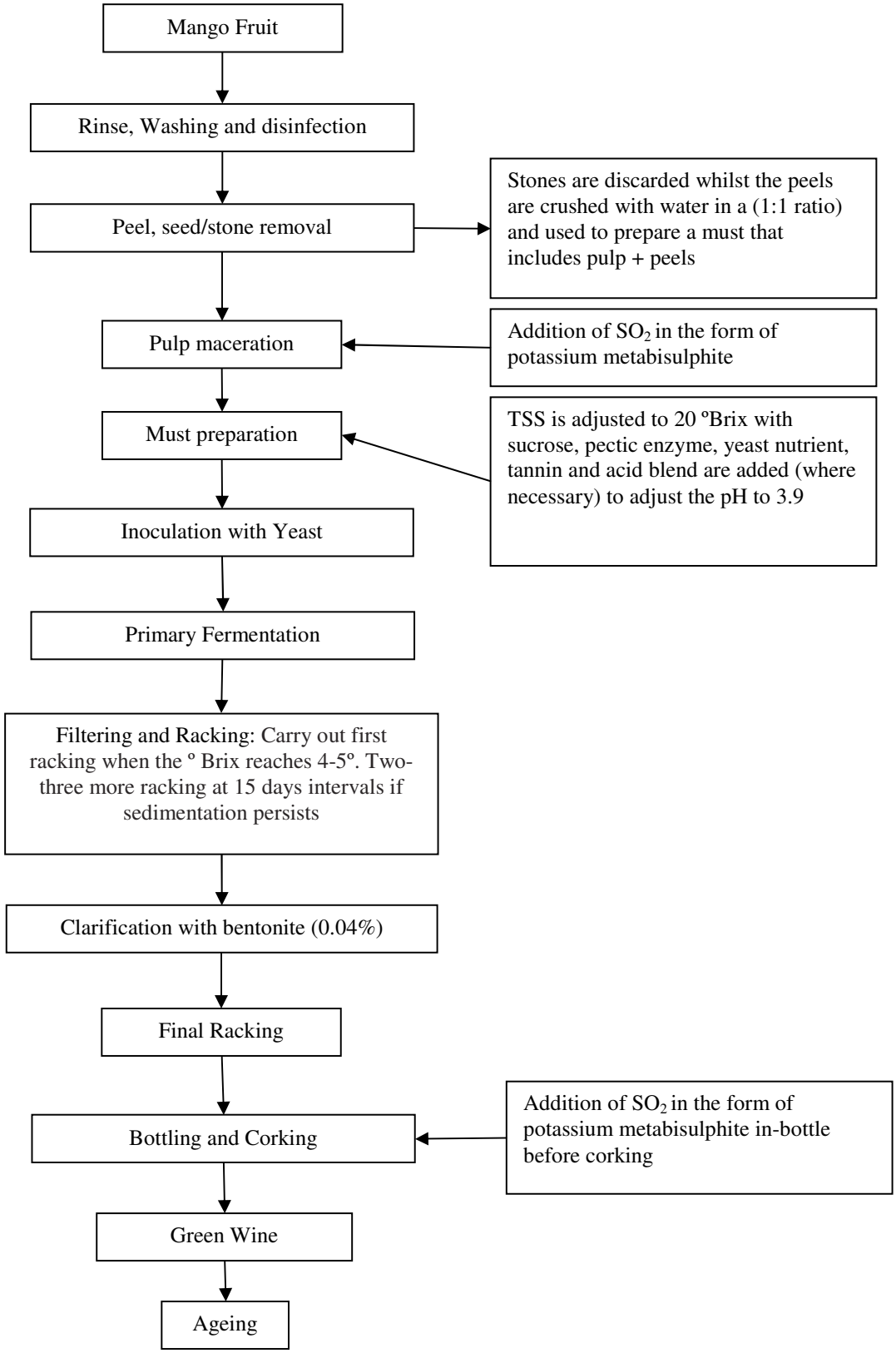


Figure 1: Flow diagram for beverage production

2.6 Microbiological Analyses

Enumeration of aerobic mesophiles was carried out on Plate Count Agar (PCA, Merck 5463, Darmstadt, Germany) and incubated at 30^{o}C for 3 days (OIV, 2011).

Lactic Acid Bacteria (LAB) micropopulation was enumerated on deMan Rogosa and Sharpe medium (MRS Oxoid) (deMan *et al*., 1960) and incubated anaerobically in an anaerobic jar at 30^{o}C for 5 days (OIV, 2011). Yeasts were enumerated on Oxytetracycline-GlucoseYeast Extract Agar (OGYEA, Oxoid CM0545) containing 4 ml oxytetracycline (Oxytetracycline Selective Supplement Oxoid) and incubated at 25^{o}C for 5 days (OIV, 2011). Acetic Acid Bacteria were enumerated on Glucose Yeast Extract Calcium Carbonate (GYC) Media containing 12.5 mg/L of penicillin to eradicate the growth of lactic acid bacteria, after autoclaving and just before use (OIV, 2011)

2.7 Sensory Analyses

The mango wine samples obtained after racking and ageing were subjected to sensory analysis. A screening test, using the triangle test procedure, was done to select suitable panelists from a group of 30 (gender: 21 men: 9 women; age group: 22 - 43) to be used in subsequent descriptive assessments. Panelists were selected largely from graduate students and staff from the University of Ghana, Legon community based on their familiarity with fruit wines and similar beverages. Evaluation of attributes (clarity, colour, aroma, taste, palate fullness, alcohol strength and aftertaste) was done using a 5 point Hedonic scale (1 = very good, 3 = fair, and 5 = very poor) by 19 panelists (gender: 13 men: 6 women) obtained from the previous screening test. Panelists also scored the intensity of each attribute on sensory ballot sheets and indicated their overall acceptance for the beverages. Four samples per panelist were served in clean transparent cups which had been labeled with a 3-digit random number. Prior to evaluation, a session was held to familiarize panelists with the product. Questionnaires and water for mouth rinsing between each tasting were provided. Panelists were asked to read through the questionnaires and the meaning of each attribute was explained to the panelists to avoid any misinterpretation.

The beverages were presented to the trained panel and the sensory evaluation data were presented as means of the panelist's score. Analysis of variance was used to test for the statistical significance of the differences observed between the scores of the beverages.

2.8 Statistical Analyses

The data were analyzed using Analysis of Variance (ANOVA) procedures in the StatGraphics Plus software for Windows Centurion version 15 (Graphic Software System, S.T.C.C., Inc., Rocksville, Maryland, USA). Significance was set at $p<0.05$ for all analyses. Pearson correlations among critical indices were determined.

3. Results and Discussion

3.1 Sugar utilization by yeast types

The rate of decrease in total soluble solids (as °Brix) during the must fermentation differed significantly ($p<0.05$) with yeast type as the fermentation time progressed, from an intial 20° Brix (Figure 2). In the first 24 hours of fermentation musts inoculated with the Lalvin D-47 (LLV) and Anchor® Brewer's Yeast (BYM) cultures showed the steepest rate of decline in sugar (°Brix) content, while musts containing Red Star® Pasteur Champagne (RSP) and Red Star® Montrachet (RSM) showed a much slower rate of change in °Brix. Similar patterns of sugar utilization by different yeast strains have been reported by Akinwale (1999), Okunowo *et al.* (2005) and Reddy and Reddy (2009) using orange, cashew and mango as fermentation substrates respectively.

The decrease in the sugar concentration during fermentation is mainly attributed to the breakdown by the yeasts and other sugar requiring organisms that may be present (Ribéreau-Gayon *et al.,* 2006). At the end of 96 hours of fermentation there were no significant differences between the °Brix content of must inoculated with the yeast types, even though BYM showed the highest residual soluble solids of 5.2°Brix while LLV had the lowest residual °Brix of 4.9. Sugar utilization along with a number of other important processes in the yeast cell is governed by the regulation of a complex array of genes that are turned off and on in order to cope with a number of stress situations (Schmidt, *et al.,* 2006). The genetic modulation apparatus differs from one strain or species of yeast to another and hence accounts for the different responses that are observed when different strains of yeasts are subjected to similar stresses (Schmidt, *et al.,* 2006).

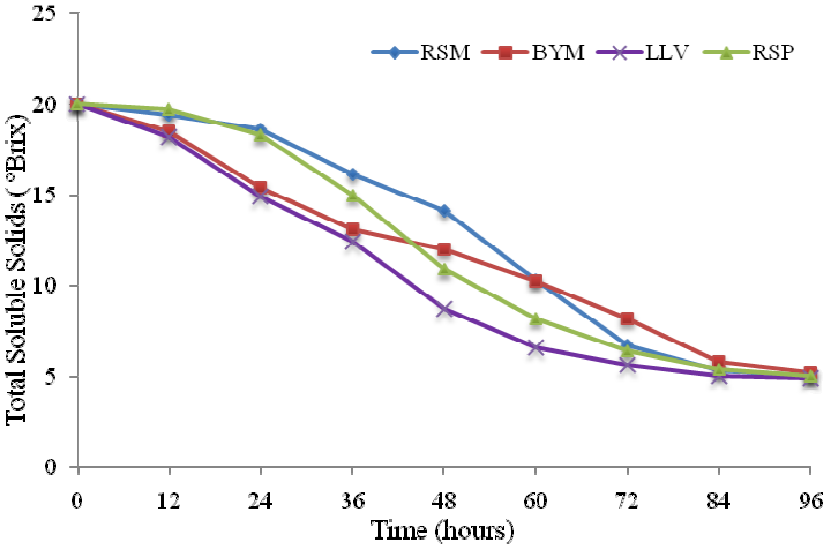


Figure 2 Pattern of sugar utilization by yeast strains over the wine fermentation period.

3.2 Change in pH and total acidity during fermentation assays

Significant changes ($p<0.05$) in pH among yeast types were observed as fermentation progressed (Figure 3). Generally, the rate of decline in pH of the musts inoculated with BYM and RSP yeast types was greater than for LLV and RSM. Over the course of the fermentation (i.e. up to 96 hours) the average rate of pH change was highest in the must inoculated with BYM followed by RSP. The rate of decline in pH by yeast type closely correlated with the rate of sugar utilization by yeast type.

The steady decline in pH during fermentation of the musts coincided with a steady and significant ($p<0.05$) rise in the total acidity over the same period (Figure 3 and 4). Similar trends have been reported by Okunowo *et al.* (2005) and, Akinwale (1999) in different fruit substrates. Metabolites such as organic acids (acetic acid, formic acids, lactic acids), phenolic compounds, esters and carbon dioxide all play a role in lowering the pH and increasing the total acid content of the must over the course of the fermentation (Onwuka and Awam, 2001; Ribéreau-Gayon *et al.*, 2006). Generally pH and acidity influence the taste of wines by imparting sour tastes to the product.

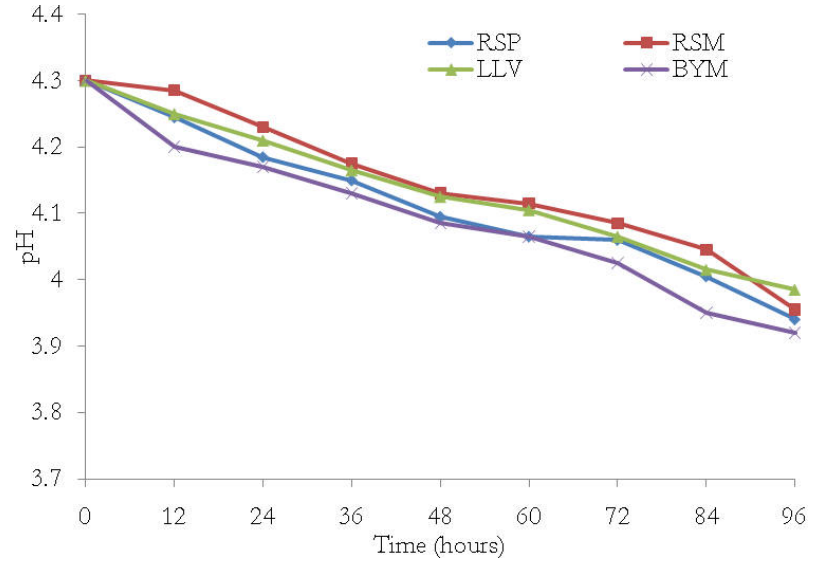


Figure 3. pH as a function of fermentation time and different yeast strains

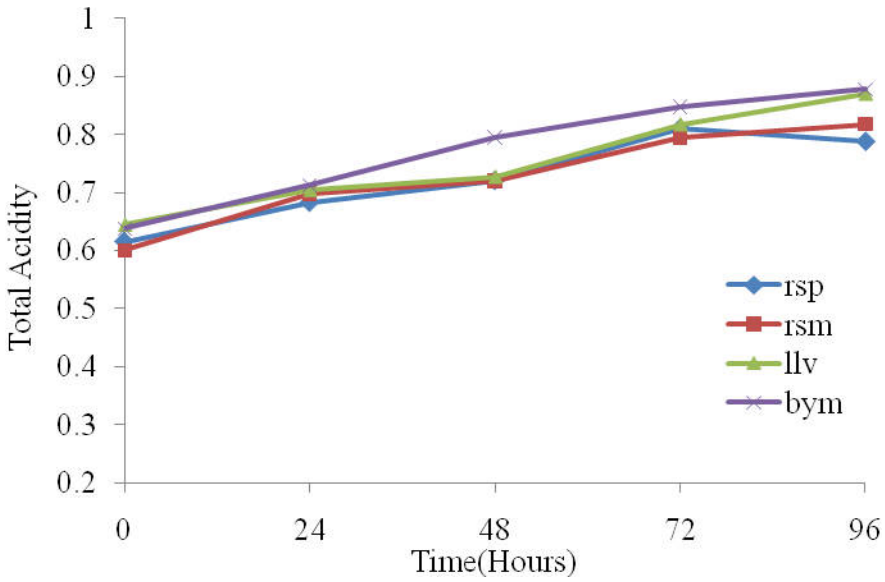


Figure 4. Total acidity as a function of fermentation time and yeast strain

3.3 Microbial population changes in fermentation assays

The total microbial population rose with fermentation time and differed significantly ($p<0.05$) between fermentation by yeast types. There was a sharp increase in the total microbial population in the first 48 hours of fermentation (Figure 5). The LLV yeast type fermentation showed the steepest rise during the first 48 hours. The total microbial population in the must inoculated with this yeast type rose from 5.62 log cfu/ml to 9.90 log cfu/ml in 24 hours. In the next 48 hours of fermentation the microbial populations in musts inoculated with LLV and RSP yeast types remained relatively stable while that of the BYM showed a slight decline from 9.31 log cfu/ml to 8.74 log cfu/ml. During the same period however, there was a marginal increase in the microbial population of the RSM yeast type from 8.74 log cfu/ml to 9.96 log cfu/ml. There was a significant ($p<0.05$) change in the aerobic mesophile population with time and with yeast type during fermentation of the mango must. The steepest rise in aerobic mesophile population occurred in the first 48 hours of fermentation.

The acetic acid bacteria (AAB) population significantly changed with fermentation time but not with yeast types. For all the fermentation runs, there was a sharp increase from a minimum (log cfu/ml) of 1.32, 1.40, 1.43 and 1.52 in the BYM, LLV, RSP and RSM fermentation assays respectively to a (log cfu/ml) acetic acid bacteria population of 3.78, 3.59, 3.52 and 3.64 in bacterial counts in the first 48 hours followed by a gentler rise in the AAB population in the next 48 hours of fermentation. Acetic acid bacteria are widespread in the winemaking environment (Joyeux, *et al.,* 1984). The population of AAB is an important consideration in winemaking because they are known to be responsible for wine spoilage due to their ability to convert ethanol to acetic acid, which leads to acidification of the wine and its negative impact on the sensory perception of the wine (Du Toit *et al.*, 2006). The levels of acetic acid bacteria found in this study were similar to those reported by Du Toit and Lambrechts (2002) and were not believed to impact negatively on the quality of the beverage produced.

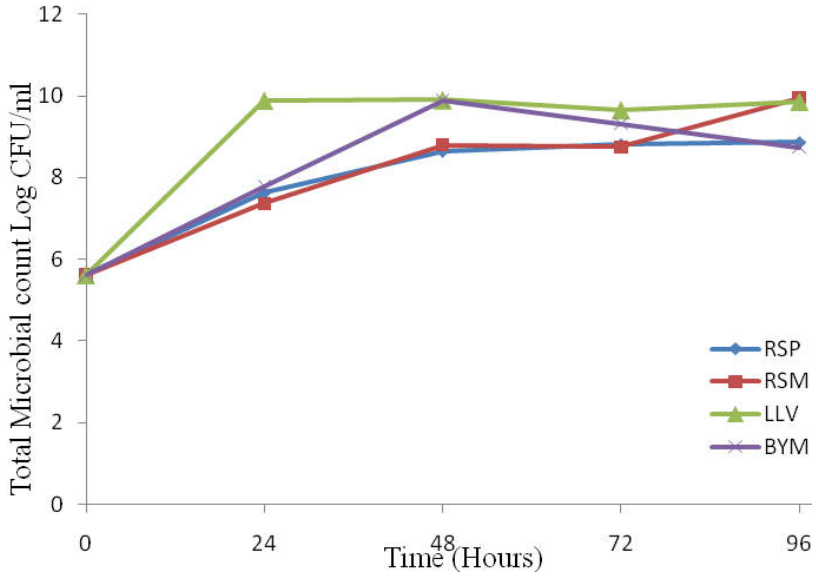


Figure 5. Changes in total microbial counts in fermentation assays inoculated with different yeast types.

3.4 Alcohol production patterns in mango must

The mango musts were made to an initial soluble solids (sugar) content of 22 °Brix and allowed to ferment to completion. Alcohol production by the yeast strains was one of the main criteria used in selecting a suitable yeast culture in fermentation. Results on the alcohol content of the wines are represented in Figure 8. Yeast type had a significant effect ($p<0.05$) on the alcohol production rate. The alcohol content was highest (11.64% by volume) when the must was fermented using yeast type RSP as the inoculum while musts fermented using yeast type BYM had the lowest alcohol content (10.15% by volume). The pattern of alcohol production did not differ significantly between the yeast type RSM and RSP on one hand and RSM and LLV on the other (Figure 6). The alcohol production in all fermentations assays increased significantly ($p<0.05$) with time, and the highest content was observed at 96 hours for all fermentations.

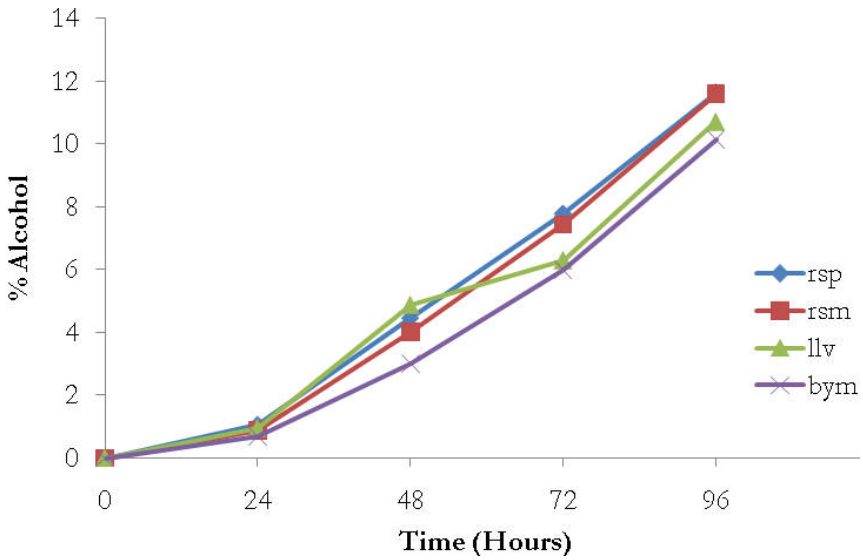


Figure 6: Alcohol production pattern in four mango fermentation assays using four different yeast types

3.5 The influence of yeast types on colour during fermentation

There was generally a decrease in the L* value (i.e. the 'lightness' value) as the fermentation progressed with time. Significant differences between the L* values of products fermented with the different yeast types were observed. The typical yellow pigment of mango results from the presence of different phenolic compounds in the fruit (Monagas and Bartolomé, 2009). These compounds are principally located in and extracted from the peel of the mango fruit. Over the course of fermentation the reactive nature of phenolic compounds, the anthocyanin equilibria, and other external and internal conditions of the fermentation (i.e. temperature, oxygen, pH, sodium metabisulfite, etc) affect the colour stability. The L* value of the fermented products from all yeast types at 48 hours showed a similar decline with mean values of musts fermented by RSP, RSM, LLV and BYM yeast types being (57.46 ± 2.73), (56.23 ± 0.23) , (58.08 ± 0.07) and (59.07 ± 0.03) respectively. The overall decline in the L* value was more pronounced in RSP (65.67±0.08 to 54.21±0.27) and least pronounced in LLV (66.73±0.06 to 59.59±0.13) after 96 hours of fermentation.

The a* value refers to the red/green colour difference in the samples. There were significant ($p<0.05$) differences between the means of the a* values of the products made using the different yeast types. The products could be classified into two homogeneous groups with RSP, BYM and LLV belonging to one group, (which were not different in their a* values) and RSM to another group. The largest change in a* value was observed in the must that was fermented using the RSP yeast type. Overall, the RSP fermentation assay showed the greatest increase in the a* (i.e. red shade) in the fermenting must with fermentation time.

The b* value denotes a yellow/blue shade. A positive value for b* indicates that the sample would appear in the yellow region of the colour space. If negative, the sample would appear in the blue region. There were positive values for b* in the products fermented using the various yeast types, giving an indication of a yellow shade in all of them. Multifactor analysis of the means of the b* revealed that yeast type had no significant effect on the b* values while fermentation time significantly impacted the b* value. There was a significant decline in the b* value (yellowness) with fermentation time in all the must fermented using the four different yeast types.

3.6 Sensory analyses of beverages produced with various yeast types

Mango wine samples produced using the local (wild) mango pulp and four different yeast types, were aged for 8 weeks and presented to a previously screened sensory panel (n=19). The product attributes evaluated during the sensory tests were taste (sweet to sour), colour (pale yellow to brown), aroma (weak to strong), clarity (clear to hazy), mouth-feel or palate fullness (thin to full), alcohol strength (low to high) and aftertaste (mild to harsh) using line scales to score intensity of attributes (Table 1). The hedonic scale (1 to 5) was also used to score consumer preference (Figure 7).

The samples were judged to have a clarity range between 1.3 to 2.7. Samples made using the RSP, LLV and RSM yeast types did not significantly differ ($p<0.05$) from each other in clarity. Samples made using the RSM and BYM yeast cultures also did not differ significantly from each other (Table1).

Analysis of colour data showed that the panelists did not find the products made using yeast types BYM (5.75 ± 2.16) and LLV (6.28 ± 2.17) to be significantly different. On the other hand, the mean scores by panelists for the color of beverages made using yeast types RSP (1.2 ± 0.79) and LLV (2.37 ± 0.68) were significantly ($p<0.05$) different from each other, even though both had a pale yellow color.

Some of the factors that influence the colour development during fruit wine fermentation include concentration of sulphur dioxide and pH of the wine (which affect the extent to which pigment compounds are bleached), the anthocyanin content, the contribution of tannins extracted from the fruit pulp and skin, polymerisation reaction kinetics, enzymatic browning reactions that may occur and the oxidation of pigment compounds present in the substrate (Ribéreau-Gayon *et al.,* 2006). The yeasts used in fermentation do not only have the ability to affect the fermentation rate and the nature and quantity of secondary products formed during alcoholic fermentation but also influence the aromatic characters of the wine (Ribéreau-Gayon *et al.*, 2006). Panelists could not differentiate between beverages produced using the RSM and BYM yeast types, and both were rated to have a strong aroma (score above 5). The aroma of LLV (4.32) did not differ significantly from the other samples. Though the RSP-fermented beverage (wine) was rated slightly sour (6.01) by panelists, it was not different from the other samples in terms of sourness. The LLV and BYM fermented products were not different from each other in taste and were judged by the panelists as sweet (Table 2). The mouthfeel of RSP-fermented and RSM-fermented beverages were judged to be slightly full (5.2) and thin (3.5) respectively.

The sensation of aftertaste is noted at a predetermined time after completion of tasting, usually 1 min (Keane, 1992). Although aftertaste was not significantly different between yeast types employed, it was scored averagely

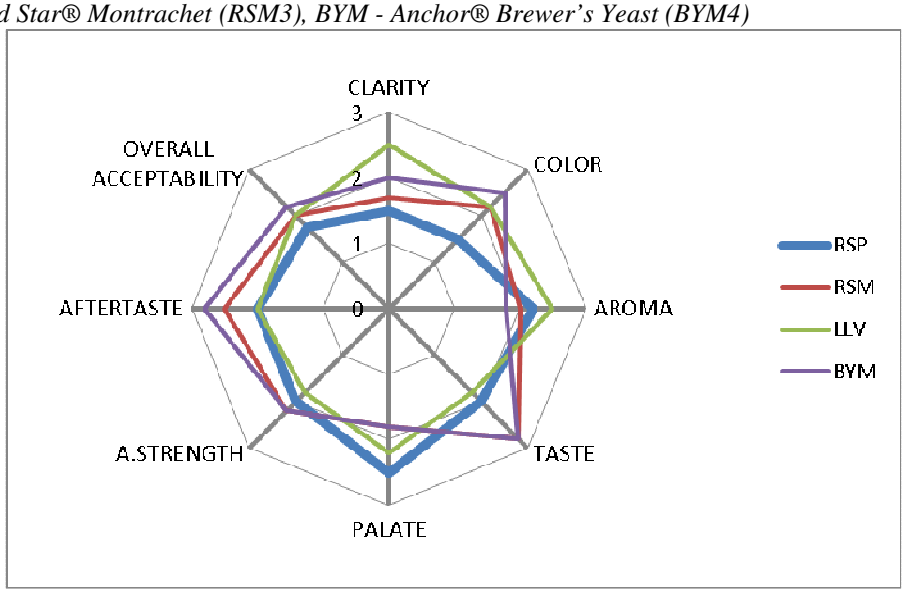
between 5.84 and 6.98. This suggests that most panelists thought all the beverages had a hint of harshness in their aftertaste.

Affective sensory evaluation of the beverages produced using the four yeast types revealed a greater preference for products made using yeast types RSP and LLV based on alcoholic strength, taste and aftertaste of the beverages. In terms of clarity, colour, aroma and palate fullness the panelists showed the greatest preference for the RSP. Figure 7 shows that the RSP yeast type has the best scores for clarity, color, taste and overall acceptability. The worst is BYM.

Table 2 Summary of mean attribute intensity scores for beverages produced with four (4) yeast types

Sample*	Clarity	Colour	Aroma	Taste	Palate Fullness	Alcohol strength	Aftertaste
RSP	1.30±1.00[a]	1.20±0.79[a]	3.67±2.18[d]	6.01±2.00[c,d]	5.29±1.91[a]	6.10±2.05[b,c]	6.18±1.71[a]
RSM	2.03±1.49[a,b]	5.76±2.16[b]	5.12±2.36[e]	5.42±2.07[c]	3.50±1.71[c]	5.07±2.34[c]	5.98±1.71[a]
LLV	1.80±1.31[a]	2.37±1.68[c]	4.32±2.22[d,e]	4.74±2.23[c]	4.57±1.81[a, c]	6.57±1.78[b]	5.84±2.17[a]
BYM	2.75±1.86[b]	6.28±2.17[b]	5.64±1.47[e]	6.82±1.54[d]	4.47±2.80[a,c]	5.39±2.19[b,c]	6.72±1.57[a]

[a, b, c] *Values with different superscripts in the same column are significantly different (P < 0.05).*
**Sample Codes: RSP - Red Star® Pasteur Champagne (RSP1); LLV - Lalvin D-47 (LLV2);*
RSM - Red Star® Montrachet (RSM3), BYM - Anchor® Brewer's Yeast (BYM4)



Scale: 1 = Very good, 2 = Good, 3 = Fair, 4 = Poor, 5 = Very
**Sample Codes for yeast types: RSP - Red Star® Pasteur Champagne (RSP); RSM - Red Star® Montrachet (RSM), LLV - Lalvin D-47 (LLV); BYM - Anchor® Brewer's Yeast (BYM)*

Figure 7. Mean attribute preference rankings for wines produced with four (4) yeast types

4. Conclusion

The type of commercial yeast used in the fermentation of the mango must was important as it influenced the color, clarity, taste, aftertaste and overall acceptability of the mango wine. The yeasts types differed considerably in fermentative capabilities and the Red Star® Pasteur Champagne (RSP) yeast produced the most preferred wine with acceptable sensory properties.

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