

The Physico-chemical Characteristics of Yeast Fermentation of two Mango (*Mangifera indica* Linn) Varieties.

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Abstract

Efficient methods of post harvest handling, preservation and value addition are critical for minimizing high losses in the post harvest chain of fruits. The goal of this study was to address this problem by employing yeast fermentation technology to produce a more stable, value added product from mangoes. The design of the study involved determination of the fermentative capabilities of a selected yeast strain on the quality characteristics of mango wine obtained from two selected mango cultivars (improved and wild) with and without peels. The response variables monitored in the must and wine included total soluble solids (TSS), pH and total acidity (TA), microbial populations (aerophilic mesophiles, yeasts and Acetic acid Bacteria), and alcohol content. Volatile compounds development was also monitored using GC-FID procedures. Descriptive and hedonic sensory evaluations were carried out on the mango wine obtained from all treatments. The effects of mango peels in must fermentation characteristics compared well with those of must fermented without peels. However, the wines made using peeled mangoes were far more preferred by consumers than wine made using mangoes with peels. Five (5) major classes of aromatic volatiles were identified in all must and wine samples. Acetaldehyde and ethyl caprylate were present in all treatments, followed by isobutyraldehyde and 2, 3 Butanedione. Some volatiles identified appeared to be mango cultivar specific (Benzaldehyde and 1-methyl-2-pyrrolidone) while other volatiles appeared to be unique to the yeast strain employed (Ethyl butyrate).

Keywords: mango, peels, yeast, must, wine, volatiles, alcohol

1. Introduction

Current global interest in mangoes has transformed them into international items of commerce with huge export earning potential (Tharanathan *et al.*, 2006; Evans, 2008). In Ghana several cultivars of mangoes are cultivated, but the Keitt cultivar is by far the most popular, and accounts for approximately 85% of mango under cultivation (Asamoah, 2006). However because of management challenges, stringent restrictions in international trade, as well as short shelf life of 2–4 weeks at maturity very high losses occur along the mango post harvest chain (Ameyapoh *et al.*, 2010). Consequently, processing mangoes into high value, shelf-stable products is essential in the management of the cyclical production gluts and the ensuing post harvest losses. Efficient processing technologies will also offer prospects of diversifying the scope of utilization and markets for the fruit (Gitonga *et al.*, 2010).

A viable method for processing and preserving mango is to ferment the juice, which has high sugars content into wines. Many studies have demonstrated the feasibility of using tropical fruits, such as banana (Onwuka, and Awam, 2001), cacao (Dias *et al.*, 2007 and Duarte *et al.*, 2010a), cashew (Akinwale, 1999; Mohanty, *et al.*, 2006; Silva *et al.*, 2007), pawpaw or papaya (Lee *et al.*, 2010), pineapple (Pino and Queris, 2010), mango (Reddy and Reddy, 2005; Pino and Queris, 2011) and orange (Selli *et al.*, 2003) to produce alcoholic beverages. Alcoholic (wine) fermentation by yeasts involves converting fermentable sugars present in fruit musts into alcohols, esters, and other volatile and non-volatile components (Duarte *et al.*, 2010b). At maturity, both reducing and non-reducing sugar content are high, and soluble sugars present in the mango pulp consist mainly of glucose, fructose, and sucrose (Tharanathan *et al.*, (2006), and can be used by yeasts as fermentable sugars. Li *et al.*, (2011) compared the chemical and volatile composition of mango wines fermented with three *Saccharomyces* strains and concluded that it was possible to make mango wine with different characteristics using different *Saccharomyces* strains. Besides yeast selection, another known factor affecting wine flavor is fruit variety. This study investigated the effect of mango peels on the physico-chemical characteristics, volatile compounds and sensory acceptability of wine made from two different mango cultivars.

2. Materials and Methods

2.1. Sample Collection and preparation

Two cultivars of mango fruit were used in the study. Ripe matured fruits of an improved mango cultivar (Keitt) and the local (wild) variety were obtained from mango growers in the Greater Accra and Volta Regions

respectively. The fruits were rinsed in clean potable water and disinfected by submerging in potassium metabisulphite solution (350ppm) for one (1) hour, rinsed, and then air-dried.

2.2. Juice Extraction and must preparation

Two types of juices were extracted from each mango cultivar: juices were extracted using unpeeled fruit, and fruit with the peels removed. In either case the fruit was macerated into pulp in a warring blender. Potassium metabisulfite was added to the macerated pulp at a concentration of 100 mg/l to inhibit bacterial growth. Pectinase (BSG HandCraft, USA) at a concentration of 4g/l was added to the must, stirred then left overnight at $22 \pm 2^\circ\text{C}$. The brix was determined and sucrose added to the pulp to adjust the total soluble solids content to 22 °Brix. Other additives such as yeast nutrient (Diammonium Phosphate and Ammonium Phosphate), tannin powder were added. The pH of the must was adjusted to 3.9 by adding a mixture of organic acids where necessary.

2.3. Fermentation of musts

Six (6) litres of mango must was transferred into fermentation vessels, fitted with anaerobic glass air-locks for anaerobic fermentation. The must was pitched with Red Star Pasteur (RSP) Champagne yeast (Fermentis, a Division of S.I. Lesaffre Group – USA) and allowed to ferment to a stable total soluble solid content at room temperature (22°C). During the anaerobic fermentation phase the wine was racked every two weeks when total soluble sugars (TSS) dropped to 4-5 °Brix without a further drop occurring for the next 48 hours. Racking was done by siphoning the fermented liquor (supernatant) into another clean vessel. Two or three more rackings were done at 15 days interval to remove any sediment deposited in the beverage where necessary. After three months of bulk ageing, the wine was transferred into bottles and stored under refrigeration ($10\text{-}15^\circ\text{C}$) for 3 months.

2.4. Physico-Chemical Analyses

Percent Total Soluble Solids (as °Brix) of the samples was determined using a refractometer (AOAC, 1990). The pH of the slurry was determined using a pH/conductivity meter equipped with a temperature sensor (OAKTON deluxe water proof pH/ Conductivity meter kit, model No. 35630-62). The total acidity was determined (AOAC 1990) and calculated as percent citric acid while volatile acidity was calculated as percent acetic acid (Saritha *et al.*, 2009). The alcohol content (by volume) was determined according to the method described in AOAC (1990).

2.5. Volatile Compounds Analyses with GC-FID

Volatile compounds in the fresh and fermented must were analyzed according to a protocol described by Duarte *et al.*, (2010b) with some modifications. The beverages were analyzed directly without any pre-treatment and cleanup procedures. A Varian CP-3800 gas chromatograph equipped with a Split/Splitless injector, a flame ionization detector, and a capillary column (30 m x 0.25 mm i.d., 2.5 μm film thickness; Chrompack) coated with CP-Wax 52 CB was used. The temperature of the injector and detector was set to 250°C . The oven temperature was held at 40°C for 5 min, then programmed to run from 40°C to 260°C at $10^\circ\text{C min}^{-1}$, and then held at 260°C for 3 min. Nitrogen was used as the carrier gas at a constant flow rate of 2.0 mL min^{-1} . Injections of 1 μL were made in the splitless mode (vent time, 60s). The volatile compounds were identified by comparing the retention times of the samples with those of standard compounds. Quantification of volatile compounds was performed with Varian Star Chromatography Workstation software (Version 6.41).

2.6. Sensory Analyses

The wine obtained after bottle ageing was subjected to sensory analysis. A panelists' screening test, using the triangle test procedure was employed to evaluate the ability of panelists to detect differences in two types of white wine. This was done to select suitable panelists from a group of 30 (aged 22 - 43) for subsequent descriptive assessments of wine samples. The panelists were selected largely from among graduate students and staff of the Department of Nutrition and Food Science of the University of Ghana based on their availability and familiarity with fruit wines and similar beverages. A total of 19 suitable panelists (comprising 13 men and 6 women) were obtained from the screening tests, and used to evaluate the sample wine attributes. The attributes of interest included wine clarity, colour, aroma, taste, palate fullness, alcohol strength and aftertaste, using a 5 point Hedonic scale where: 1 = very good, 3 = fair, and 5 = very poor.

The panelist 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 pre-labeled with a 3-digit random number. Questionnaires and water for mouth rinsing between each tasting were provided. Prior to evaluation, a session was held to familiarize panelists with the product and the questionnaire.

2.7 Experimental design and data analysis

Juices obtained from macerating two mango cultivars (Keitt and local) with and without peels were pitched with a known yeast strain (RSP) and fermented into wine following basic standard wine making protocols, in a 2 x 2 factorial design (mango cultivar X juice with and without peels). Product indices of pH, titratable acidity, °Brix, color and alcohol content were monitored. Sensory evaluation of the wine was done using a trained panel of 19. The data were analyzed using analysis of variance (ANOVA). Significance was set at $p < 0.05$ for all analyses.

3. Results and Discussion

3.1 Physicochemical characteristics of two mango cultivars

The physicochemical indices of the two mango cultivars i.e. *Mangifera indica* (cv. Keitt) and the local cultivar of mango are listed in Table 1. Fruit of the Keitt cultivar was generally larger and weighed far heavier than the local cultivar. In terms of yield, the Keitt mango fruit also yielded more pulp (74.6%) than the local cultivar (64.4%). The seed of the local variety accounted for about 13.4% of the total mass of the fruit while that of the Keitt cultivar accounted for 7.1% of the total mass of the fruit. The contribution of the peels to the overall weight of the fruits was 17.5% and 20.4% for the Keitt cultivar and the local cultivar respectively. Peel colour of the mature Keitt fruits was different (green to pale yellow) while that of the local variety of mango was uniformly yellow. The total soluble solids content was higher in the Keitt cultivar than the local mango cultivar (Table 1). The total acidity and volatile acidity of the local cultivar was higher than that of the Keitt cultivar and the vitamin C content of the Keitt was also lower than that of the local cultivar.

3.2 Sugar utilization patterns in musts from both the local and Keitt varieties

There were no significant differences ($p < 0.05$) between mango varieties in the sugar utilization profile during fermentation (Figure 1). Sugar utilization by yeasts on the must of the local mango cultivar (LPM) and the Keitt cultivar (KPM) declined starting from 20 to 5.1 °Brix and 20 to 4.7 °Brix respectively in 96 hours of fermentation. However, the presence of mango peels significantly affected the sugar utilization rate. Must prepared from (Keitt) mango with the peels removed (PON) showed a steeper decline in soluble solids (°Brix) than the must prepared from mangoes with the peels on (PNP) (Figure 2). At the end of 96 hours of fermentation the PNP assay showed a marginally higher total soluble solid (TSS) content (°Brix) of 5.2 than the PON assay of 4.9 °Brix.

Table 1 Physicochemical characteristics of Mango varieties

| CHARACTERISTIC | MANGO VARIETY | |
|---|----------------|---------------|
| | KEITT | LOCAL |
| Total weight of fruit (g) | 1020.8 ± 111.6 | 186.1 ± 27.7 |
| Pulp (g) | 762.0 ± 3 | 119.9 ± 2.8 |
| Peel (g) | 179.2 ± 5.5 | 38.1 ± 2.4 |
| Seed (g) | 72.5 ± 3.3 | 25.1 ± 3.2 |
| Juice Yield (ml/kg) | 714.3 ± 15.1 | 649 ± 7.4 |
| Length (mm) | 155.5 ± 3.7 | 74.7 ± 2.7 |
| Largest Circumference (mm) | 364 ± 29.6 | 193 ± 5.8 |
| Sugar (g/L) | 167.6 ± 1.7 | 139.4 ± 8.2 |
| Total soluble solids (°Brix) | 16.4 ± 0.16 | 13.8 ± 0.14 |
| Specific Gravity | 1.0702 | 1.0567 |
| Total acidity (% anhydrous citric acid) | 0.478 ± 0.019 | 0.578 ± 0.019 |
| °Brix : Acidity Ratio | 34.30 | 23.58 |
| pH | 4.44 ± 0.04 | 3.94 ± 0.18 |
| Vitamin C content (mg/100g) | 28.87 ± 0.74 | 43.62 ± 0.9 |
| Pulp Colour (L*) | 73.54 ± 0.18 | 61.71 ± 0.07 |
| Pulp Colour (a*) | 0.22 ± 0.02 | -2.43 ± 0.12 |
| Pulp Colour (b*) | 51.85 ± 0.32 | 68.09 ± 0.17 |

3.3 pH and total acidity

The trend of pH changes during fermentation by mango variety is depicted in Figure 3. A drop in pH from 4.3 to 3.82 and from 4.3 to 3.66 for local mango pulp (LPM) and Keitt mango pulp (KPM) respectively occurred over the course of 96 hour period. The pH of both mango musts declined steadily from 4.3 ± 0.01 in the first 48 hours of fermentation. Reddy and Reddy (2005) reported similar differences in pH and total acidity of wines produced from different mango cultivars with similar starting total soluble solid contents. The inclusion of peels during must preparation also significantly influenced the pH (Figure 3) and total acidity. Over the course of the fermentation the PNP showed a higher rate of increase in total acidity, while the PON displayed a more modest increase over the period.

3.4 Changes in total microbial count and yeast population during fermentation

The total microbial and yeast population counts in musts of the two mango varieties were significantly ($p < 0.05$) influenced by fermentation time and not mango variety. There was a greater increase in the total aerophilic mesophile count in the local (LPM) than in the improved Keitt (KPM) cultivar during fermentation.

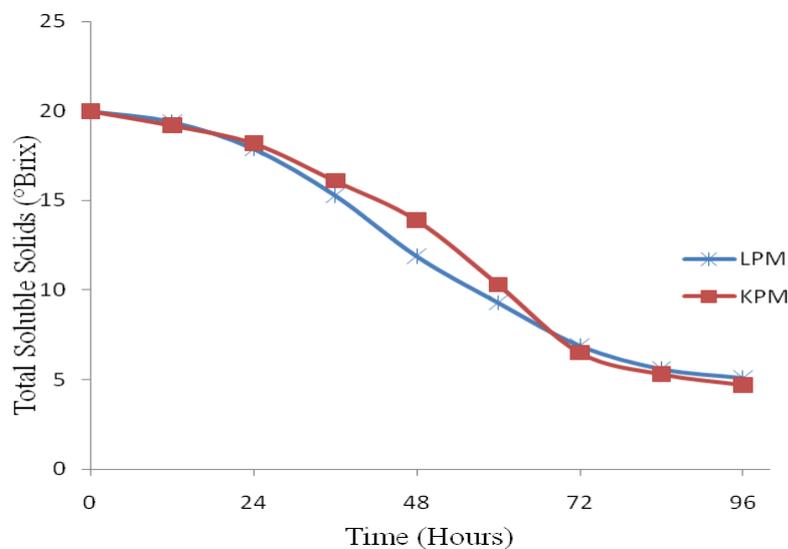


Figure 1. Pattern of sugar utilization on two mango musts over fermentation period

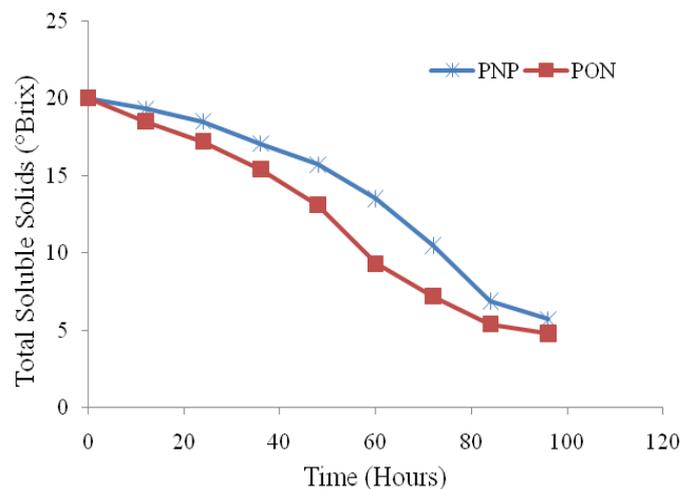


Figure 2. Pattern of sugar utilization on two mango musts (PNP and PON) over fermentation period

3.5 Alcohol production during mango must fermentation

There were significant differences between the mango cultivars and with fermentation time on alcohol content (Figure 4). Alcohol content reached a maximum of 11.54% and 11.63% for LPM and KPM respectively in 96 hours. Fermentation of must with or without peels also had some influence on alcohol content. Alcohol content in the PON and PNP assays reached 11.54% and 11.63%, respectively after 96-hours of fermentation. In terms of alcohol yield alone, fermentation of must with peels (PNP) may be considered better in the production of ethanol.

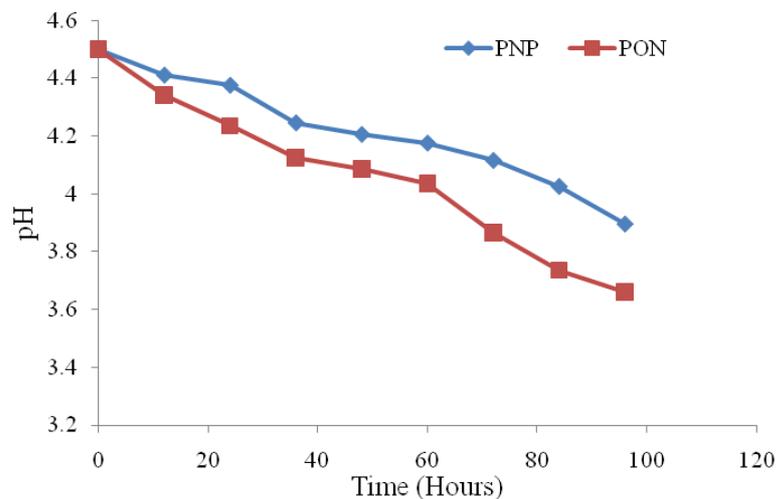


Figure 3. pH as a function of fermentation time and mango musts without (PNP) and with peels removed (PON) during fermentation.

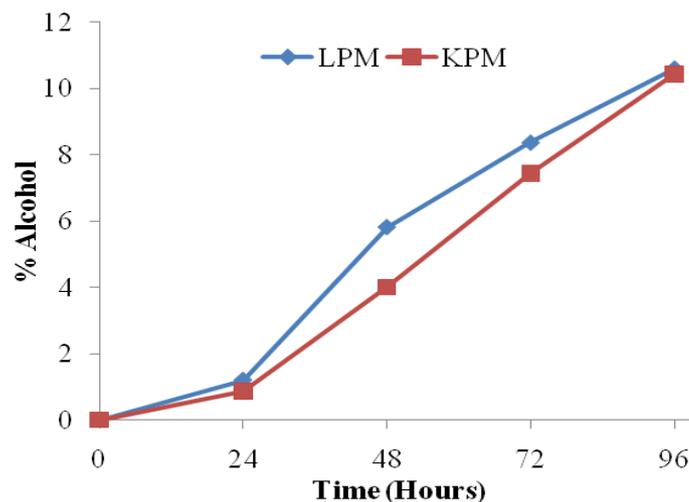


Figure 4. Alcohol content in musts during fermentation by mango cultivar

3.6 Consumer acceptability of mango wine produced from Local and Foreign mango pulps

The wines were evaluated by a previously trained panel on a 5-point hedonic scale (1 = very good, 3 = fair, and 5 = very poor) using a preference test (Taylor, 2004). There were no significant differences between the wines produced using Local Mango Pulp (LPM) and Keitt Mango Pulp (KPM) with respect to clarity, colour, aroma, palate fullness alcohol strength and aftertaste. The only attribute that showed some difference between the mango cultivars was the taste. Wine produced using Local mango pulp (LPM) was perceived to be slightly sour (5.8) compared to a sweet taste (4.3) for KPM. As shown in Table 2, products from both substrates were judged averagely as clear (2), having a pale yellow colour (3) with a high alcohol strength (6). Whilst KPM felt thin in the palate (3.9), LPM was full (4.8) on the palate but both had slightly harsh aftertaste (6) when consumed. Though there was no difference between the samples, wine made from LPM was rated as having a weak aroma (4.3) compared to strongly rated aroma (5.6) for KPM wine. The panelists liked the alcohol strength and clarity of both beverages but rated attributes such as aftertaste and palate fullness poorly. In terms of overall acceptability, the panelists preferred the beverage made from the local mango pulp.

Table 2. Summary of mean attribute intensity scores and one-way ANOVA analysis for beverages produced.

| Sample* | Clarity | Colour | Aroma | Taste | Palate Fullness | Alcohol strength | Aftertaste |
|---------|-------------|-------------|-------------|-------------|-----------------|------------------|-------------|
| LPM | 1.90 ± 1.03 | 2.81 ± 1.64 | 4.39 ± 2.54 | 5.85 ± 1.72 | 4.84 ± 1.72 | 6.24 ± 2.12 | 5.15 ± 1.85 |
| KPM | 1.64 ± 0.99 | 2.52 ± 1.34 | 5.61 ± 1.74 | 4.39 ± 1.65 | 3.90 ± 1.49 | 5.65 ± 2.14 | 5.67 ± 1.99 |

Significant means are denoted by different superscripts down a column and are significant at $p < 0.05$.

*Sample Codes: LPM – Beverage prepared with local Mango cultivar. KPM – Beverage prepared with Keitt Mango cultivar

Scale: 1=Very Good, 2= Good, 3 = Fair, 4= Poor, 5=Very Poor.

3.7 Identification of flavour volatiles

Eighteen (18) volatile compounds were identified in all the samples analyzed (Table 3). They included esters (5), aldehydes (3), diacetyl (1), higher alcohol (1), organic acids (3), monoterpenes (2), volatile phenols (2) and the compound 1-methyl-2-pyrrolidone.

3.7.1 Esters

Ethyl acetate was identified in the commercial wine samples, wines made from local and Keitt mango varieties, with and without peels. Ethyl caproate (Ethyl hexanoate) was identified in only the fresh pulp of the local mango variety while ethyl caprylate (Ethyl octanoate) was identified in all samples analyzed. Esters were clearly the dominant constituents in the mango wine and they can originate both from the substrate used in fermentation (Pino *et al.*, 2005; Quijano *et al.*, 2007), and can also be synthesised during the alcoholic fermentation by yeast (Nykänen, 1986; Swiegers, *et al.*, 2005). The esters identified may play a significant role in imparting distinct aroma notes. Swiegers *et al.*, (2005) attributed a fruity, solvent-like aroma to the ester ethyl acetate. The presence of ethyl acetate and ethyl caproate (ethyl hexanoate) esters in the fresh pulp and must and its subsequent absence from the fermented samples may be attributed to their utilization during the fermentations or transformation to other compounds. Ethyl caprylate (ethyl octanoate) persisted in all samples both in the fresh pulp (Lalel *et al.*, 2003) but could have also been produced in the wine by the yeast (Swiegers *et al.*, 2005) during fermentation.

3.7.2 Aldehydes

The aldehydes identified in the analysis were acetaldehyde, benzaldehyde and isobutyraldehyde. Aldehydes contribute to flavour with aroma descriptors such as 'bruised apple' and 'nutty' but can also be a sign of wine oxidation (Swiegers *et al.*, 2005). Acetaldehyde was present in every pulp treatment and every fermentation assay using the local mango pulp variety. Acetaldehyde, also known as ethanal is a major carbonyl compound found in most fermented fruit beverages. As a final precursor before ethanol production, acetaldehyde is a major metabolic intermediate in yeast fermentation. It is made from the conversion of pyruvate (the end-product of glycolysis) through pyruvate decarboxylase enzymes (Moreno-Arribas and Polo, 2009; Swiegers *et al.*, 2005). Benzaldehyde was detected in the fresh local pulp, but absent from all fermented wine samples. Its presence may be a characteristic of that pulp. Benzaldehyde was identified by a number of researchers in fresh mango pulps of other cultivars (Torres *et al.*, 2007; Quijano *et al.*, 2007; Pino and Mesa, 2006). The aldehyde isobutyraldehyde was found in the local fresh pulp and in all fermentation assays carried out with that pulp. Together, benzaldehyde and isobutyraldehyde form minor components of most wines but are linked with the mechanism for aldehyde-mediated condensation reactions which lead to the production of pigment compounds (Monagas and Bartolomé, 2009).

Table 3: Summary of volatile compounds identified in pulp, must and wine samples by GC-FID

| Compound | Pulp Treatments | | | Yeast strain on local pulp | Commercial wines | |
|------------------------|-----------------|------------|-------------------|----------------------------|------------------|------------|
| | Local Pulp | Keitt Pulp | Keitt Pulp + Peel | RSP | Red Wine | White Wine |
| Ethyl acetate | * | * | * | Nd | nd | nd |
| 2,3-Butanedione | * | ** | ** | Nd | * | * |
| Ethyl butyrate | nd | nd | nd | * | nd | nd |
| 3-Methylbutanol | nd | nd | nd | Nd | * | * |
| Ethyl caproate | * | nd | nd | Nd | nd | nd |
| Ethyl caprylate | * | * | * | * | * | * |
| Benzaldehyde | * | nd | nd | Nd | * | * |
| Isobutyraldehyde | ***** | nd | nd | * | * | * |
| Acetaldehyde | ***** | * | ** | * | * | * |
| Butyric acid | * | * | * | * | nd | nd |
| Isovaleric acid | nd | nd | nd | Nd | * | * |
| 1-Methyl-2-pyrrolidone | * | nd | nd | * | * | * |
| 2-Phenethyl acetate | nd | nd | nd | Nd | * | * |
| Geraniol | * | * | * | Nd | nd | nd |
| Guaiacol | * | nd | nd | Nd | * | * |
| Octanoic acid | nd | nd | nd | Nd | * | * |
| Eugenol | nd | nd | nd | Nd | * | * |
| Linalool | nd | nd | nd | Nd | * | * |

* Relative Intensities of volatile peaks, nd – not detected

3.7.3 Ketone-Diacetyl

The ketone 2, 3-Butanedione, commonly referred to as ‘diacetyl’, is a major flavour compound in dairy products and was present in all samples except the fermentation assay carried out. When present at a high concentration (exceeding 5–7 mg/L) in wine, diacetyl is regarded by many to be undesirable (Swiegers *et al.*, 2005). It is considered to contribute a desirable ‘buttery’ or ‘butterscotch’ or ‘nutty’ aroma depending on its concentration (Monagas and Bartolomé, 2009; Swiegers *et al.*, 2005). The formation and degradation of diacetyl is directly related to the growth of malolactic bacteria. It is formed as an intermediate metabolite in the reductive decarboxylation of pyruvic acid to 2,3-butanediol (Monagas and Bartolomé, 2009; Swiegers *et al.*, 2005).

3.7.4 Higher Alcohols

The higher alcohol 3-Methylbutanol or isoamyl alcohol was largely absent in all mango wine samples but present in all commercial wine samples. Higher alcohols (also known as fusel alcohols) are secondary yeast metabolites. They can also be produced by yeast during alcoholic fermentation of sugars, according to Monagas and Bartolomé (2009). Factors such as, ethanol concentration, fermentation temperature, the pH and composition of the must, aeration, level of solids, varietal differences, maturity and skin contact time affect the concentration of higher alcohols in the final product (Fleet and Heard, 1993).

3.7.5 Fatty Acids

None of the volatile fatty acids investigated, isovaleric acid and octanoic acid, were identified in any of the mango treatments. However the short chain fatty acid, butyric acid was identified in all fermentation assays. This compound was also absent in both white and red commercial wines.

3.7.6 Monoterpenes (Geraniol and linalool)

The monoterpene, geraniol; was identified in all but one of the mango pulp, must and wine samples. This monoterpene is usually synthesized from glycosylated precursors and its presence in almost all the mango samples analyzed may be due in part to the action of glycosidases in these samples (Ugliano and Henschke, 2009). Addition of enzymatic preparations like pectinases generally results in higher concentrations of different

classes of volatile compounds, particularly monoterpene alcohols, monoterpene polyols, norisoprenoids and benzenoids (Ugliano, 2009). Other researchers, have identified linalool in fresh mango pulp and wines of most mango varieties (Pino and Queris, 2011; Torres *et al.*, 2007; Quijano *et al.*, 2007; Pino and Mesa, 2006; Lalel *et al.*, 2003).

3.7.7 Volatile Phenols (Eugenol and guaiacol)

Guaiacol was detected in local mango pulp and most fermented samples (Table 3). Eugenol on the contrary, was largely absent from all mango samples analyzed. Pérez-Coello and Díaz-Maroto, (2009); reports that the concentration of these compounds in wine increases over the initial months of ageing and then levels off and remains virtually constant after 12 months in the barrel. The compound 1-methyl-2-pyrrolidone was also identified in all local pulps and commercial wine samples.

4. Conclusion

The local (wild) and keitt (improved) mango cultivars were not significantly different as substrates for wine yeast fermentation. Produce from both cultivars may be transformed into value added, shelf stable products using yeast fermentation technology. The presence of mango peels in the must during alcoholic fermentation affects the sensory qualities of the final product. Beverages obtained from must that were fermented with peels included were not well received by the sensory panelists. The presence of five (5) important classes of volatile aromatic compounds that contribute to aroma development at various points in fruit wine processing were identified. The volatile compounds identified were typical of fruit wines.

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