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The influence of process optimization on the fermentation profile of mango wine prepared from the Apple mango variety

Musyimi S.M[1], Sila D.N[1], Okoth E.M[1], Onyango C.A[2],Mathooko F.M[3]

[1]*Department of Food Science and Technology, Jomo Kenyatta University of Agriculture and Technology,P.O.Box.62000-00200, Nairobi, Kenya*
[2]*Taita Taveta University College, P.O.Box 635-80300, Voi, KENYA*
[3]*South Eastern University College, P.O. Box 170-90200, Kitui, Kenya*
Key words: yeast concentration, fermentation temperature, apple mango wine.

1 SUMMARY

The aim of this study was to determine the effects of temperature and yeast concentration on the fermentation kinetics and chemical properties of Apple mango fruit wine through process optimization. The physicochemical properties of Apple mango variety were determined for its suitability to produce wine. The fermentation conditions were optimized by varying temperature at 20°C, 25°C, 30°C and 35°C and the yeast concentration at 0.0065%, 0.01%, 0.05% and 0.1%. The increase in temperature and yeast concentration increased the fermentation kinetics significantly ($p<0.05$). However, at high temperature (35°C) and yeast concentration (0.1%) the sugars were not completely utilized during fermentation. At low temperature of 25°C, the alcohol yield was highest (9.44%) relative to high temperature of 35°C that gave the lowest yield (6.93%). Yeast concentration of 0.05% and fermentation temperature of 25°C gave the optimal characteristics for Apple mango wine using wine yeast (*Saccharomyces cerevisae*).

2 INTRODUCTION

Mango (*Mangifera indica L.)* is one of the most important fruits in the tropics and subtropics. It is commercially grown in more than 90 countries worldwide and is consumed both in fresh or processed form. In Kenya, it is the third most important fruit in terms of area and production for the last ten years after banana and pineapple (FAO 2005). In 2007, it was estimated that the area under mango production was 14,387 Ha with an output of 280,884 MT (MoA, 2007). In 2008, HCDA reported 250,000MT of mango production; however, this number greatly increased to 450,000 MT in 2010 (Ministry of Agriculture, MoA, 2010). This is a clear indication that mango production is expanding tremendously. Gathambiri (2009) reported a percentage post harvest loss of 45% and the main reason cited was excess fruits in the market during the peak seasons. Production of wine from mango is one of the alternative ways to use and convert surplus production into a valuable product (Onkarayya 1986; Reddy 2005). Limited research and information regarding the optimal conditions for mango wine production are available in Kenya.

Alcoholic fermentation is a combination of complex interactions involving must variety, micro biota and winemaking technology (Ribe´reau-Gayon *et al.*, 2000). Some factors strongly affect alcoholic fermentation, and consequently the quality of the wine. The most important factors are the clarification of the juice, the temperature of fermentation, the composition of the juice, inoculation with selected yeasts and the interaction with other microorganisms (Ribe´reau-Gayon *et al.*, 2000). One of these factors, the temperature of

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fermentation, directly affects the microbial ecology of the must and the biochemical reactions of the yeasts (Fleet and Heard, 1993).
There has been limited information in the research on mango wine until recently, although it started from 1960's. Czyhrinciwk (1966) reported the first study on mango wine production. Onkarayya and Singh (1984) screened twenty varieties of mangoes from India for wine production. Reddy and Reddy (2005) developed a method of mango juice extraction with pectinase and characterized ethanol and some volatile contents of mango wine. Although there are various studies on the effects of pitching levels of brewer's yeasts on beer fermentation, (Edelen, Slaughte and Suihko, 1996,1988 and 1993) information concerning the effects of the pitching level of the wine yeast *S. cerevisiae* on wine fermentation is scarce. There is still no complete profiling of chemical properties of mango wine at varying temperatures and yeast innoculum sizes although a complete profile of chemical and volatiles of fresh mango juice is available (Pino & Mesa, 2005; Pino *et al.*, 2005).
Therefore, the aim of this study was to determine the fermentation kinetics of wine yeast at varying temperatures and yeast concentrations and the chemical properties of the resultant Apple mango wine. The outcome of this study would help select appropriate temperatures and yeast concentration for further investigations involving wine yeast to enhance mango wine quality.

3 MATERIALS AND METHODS

3.1 Sample collection: Mature and healthy Apple mango fruits were obtained from a farm in Katheka Kai division, Machakos County of Kenya. They were then packed in crates and transported by a vehicle for about $1\frac{1}{2}$ hours to Jomo Kenyatta University of Agriculture and Technology, Department of Food Science and Technology. The fruits were then washed with tap water plus detergent (easy foam) and stored at an ambient temperature of 25ºC±2 to ripen. No pretreatment of the fruits was done prior to ripening. Ripeness was determined by feeling with hands for firmness and flesh colour change by observation.

3.2 Pulp extraction: Ripened mango fruits were sorted, washed and peeled manually using a knife. The flesh was cut away from the seed using a knife and then homogenized using a pulp extractor. Pulp obtained in this manner was then subjected to physicochemical analysis.

3.3 Juice preparation: After extraction, the juice was pasteurized at 65±4ºC for 10 minutes and cooled immediately with cold tap running water to 27±2ºC. The pH of the mango juice was adjusted to 4.5 by addition of calcium carbonate ($(CaCO_3)$ food grade) and citric acid ($(C_6H_8O_7)$ food grade) respectively. The mango juice was not ameliorated with fermentable sugars prior to fermentation.

3.4 Preparation of yeast culture: Active dried wine yeast obtained from Kenya Wine Agencies Limited (KWAL) was used. To determine the influence of yeast concentration on the profile of the wine, yeast inoculum sizes were varied in concentration of 0.0065%, 0.01%, 0.05%, and 0.1% sizes. Prior to inoculation, the yeast strain was rehydrated by adding it into 200ml of the mango juice at 37ºC±2 for 10 minutes. After 10 minutes, the slurry was allowed to cool and attain the same temperature as of the juice (27ºC±2) and then poured into the fermentation jars respectively.

3.5 Fermentation of the mango juice: The treated juice was divided into different portions of 500 ml and put in 1 litre sterile fermentation jars. To determine the influence of temperature on fermentation, the appropriate number of inoculated flasks as prepared previously , at different temperatures; 20°C (controller, MCU-2260C-S, Sanyo Electric Co., Ltd. Japan) 25°C, 30°C, and 35°C (Incubator IS62, Yamato Scientific Co., Ltd. Japan) were incubated and the jars shaken intermittently to evolve the dissolved CO_2 thus facilitating the fermentation process. The jars were covered using a rubber stopper fitted with a bend tube to release carbon dioxide ((CO_2)). The fermentation rate was monitored every 24 hours by checking the ºBx (brix) change.The end of fermentation was determined when the ºBx could not change any further. After fermentation, the wine samples were centrifuged (Centrifuge Model H–2000C Shimadzu Corp., Kyoto, Japan) at 7,000 rpm for 5 minutes prior to analysis. All the

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experiments were done in triplicates and the mean values determined.

3.6 Effects of temperature and innoculum size on the fermentation kinetics of Apple mango wine: The fermentation kinetics of apple mango wine were determined at varying temperatures and inoculum sizes by calculating °Bx utilization per day using first order kinetics.

$$Substrate\ \ utilizatio\ n: -\frac{d[x]}{dt} = k[x]$$

Rearrangement yields the following: $\frac{d[x]}{[x]} = -kdt$

Where x = product

t = time

3.7 Determination of effects of temperature and inoculum concentration on the chemical properties of Apple mango wine: The effects of varying temperature and yeast inoculum sizes on chemical properties of apple mango wine was determined by inoculating wine yeast at different concentrations and varying the fermentation temperatures. The resultant wine was analyzed for titratable acidity (TTA) pH, residual °Bx, alcohol content, and volatile acidity.

3.8 Analytical methods

3.8.1 Determination of juice yield: This was determined by weighing three mango fruits prior to pulp extraction and quantifying the pulp recovered after extraction as a percentage based on the weight of the samples.

3.8.2 Determination of reducing sugars: Quantification of reducing sugars present was determined using High Performance Liquid chromatography (HPLC) method as outlined in AOAC (1996). A sample of 10 g each of fruit pulp was refluxed in 96% ethanol for 1 hour. The extract was filtered using cotton wool and concentrated by rotary evaporator. This was then diluted with 75% acetonitrile in the ratio of 1:1. Standard solutions of sucrose, fructose and glucose were prepared at varying concentrations of 2mg/ml, 4mg/ml, 6mg/ml and 8mg/ml. These were injected into HPLC (LC- 10AS, Shimadzu Corp., Kyoto, Japan) fitted with the Refractive Index Detector (RID) followed by the sample extracts. The HPLC had the following conditions: oven 35°C, flow rate: 0.5-1.0 ml/min, injection volume – 20 µl, column- (NH_2P-50 E). The standard curves were drawn and used to quantify the reducing sugars of the samples.

3.8.3 Determination of pH: This was done by the method of Ofori and Hahn (1994). The pH meter used was (TOA pH Meter HM–7B, Tokyo, Japan).

3.8.4 Determination of total soluble solids (TSS): The TSS was determined using an Atago hand refractometer (RX 5000, Atago, Tokyo, Japan). The readings were expressed in ºBx

3.8.5 Determination of total titratable acidity (TTA): The TTA was determined by titrating with 0.1N NaOH (sodium hydroxide) in the presence of phenolphthalein indicator as described using AOAC (1995) method. TTA results were expressed as % malic acid which is the main organic acid in mango fruit (Ueda *et al.*, 2000).

3.8.6 Determination of residual sugars (AOAC 2000): Residual sugars were determined in°Bx using an Atago hand refractometer (RX 5000, Atago, Tokyo, Japan).

3.8.7 Determination of volatile acidity (VA): This was determined by titrating the distillates against 0.1N NaOH and the results expressed as acetic acid (g/l) as described using AOAC (2000) method.

3.9 Statistical analysis: Analyses were done in triplicates and data assessed using Genstat 12th edition by one-way analysis of variance. Duncan multiple range test was used to determine significant means. Significance was defined at $p \leq 0.05$ and the values displayed with standard deviations of the means.

4.0 RESULTS AND DISCUSSION

4.1 Determination of Apple mango variety for its suitability in wine production: The results of juice yield and chemical composition of Apple mango juice are presented in Table 1 below. The suitability of mango variety for wine production is generally screened based on juice quality. The main prerequisite character of juice for fermentation is sugar content. In mango, three types of sugars are present: glucose, fructose, and sucrose. These comprise of the reducing sugars presented in the table below. The total soluble solids of Apple variety juice determined as °Bx was 23.9±0.21% whereas the titratable acidity as malic acid ranged

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from 0.42% to 0.50% (w/v). The pH value of the juice was between 4.21 and 4.29.

Table 1: Chemical characteristics of mango juice of Apple variety

Mango variety	Juice yield (%)	°Bx	Reducing sugars (%w/v)	*p*H	Titratable acidity (%)
Apple	71.34±1.59	23.9±0.21	23.78±1.24	4.25±0.04	0.46±0.04

Values are presented as mean ± SD; n = 3

These results suggest that mango juice from Apple variety has a potential for producing good quality wine as the determined properties were within the acceptable range for wine production (Reddy, 2005).

4.2 Effects of temperature on the fermentation kinetics of apple mango wine: Fig 1 shows the effects of temperature on the fermentation kinetics of apple mango wine at 0.05% yeast concentration. At 35°C, initial fermentation rate was high although towards the end of fermentation, day 10, the rate decreased, and the sugars were not completely utilized. At 30°C and 25°C, fermentation rate was not significantly different (p>0.05) although towards the end of fermentation the sugars were not completely utilized at 30°C. Fermentation rate was slowest at 20°C although it was consistent as shown in the figure. At this temperature, the residual sugars were of the same concentration as fermentation at 30°C. Generally, high temperatures increased the rate of fermentation, but the sugars were not completely utilized. High temperatures increased the enzyme activity during the metabolic pathway therefore, increasing the rate of fermentation (Macrae, *et al.*, 1993). On the other hand, over a period, high temperatures decrease the stability of enzymes and other biomolecules therefore decreasing the enzyme activity hence the decrease in the use of available sugars (Sevda, 2011). In contrast, low fermentation temperatures, which started more slowly, consumed most of the sugars because the high yeast biomass was maintained throughout the process.

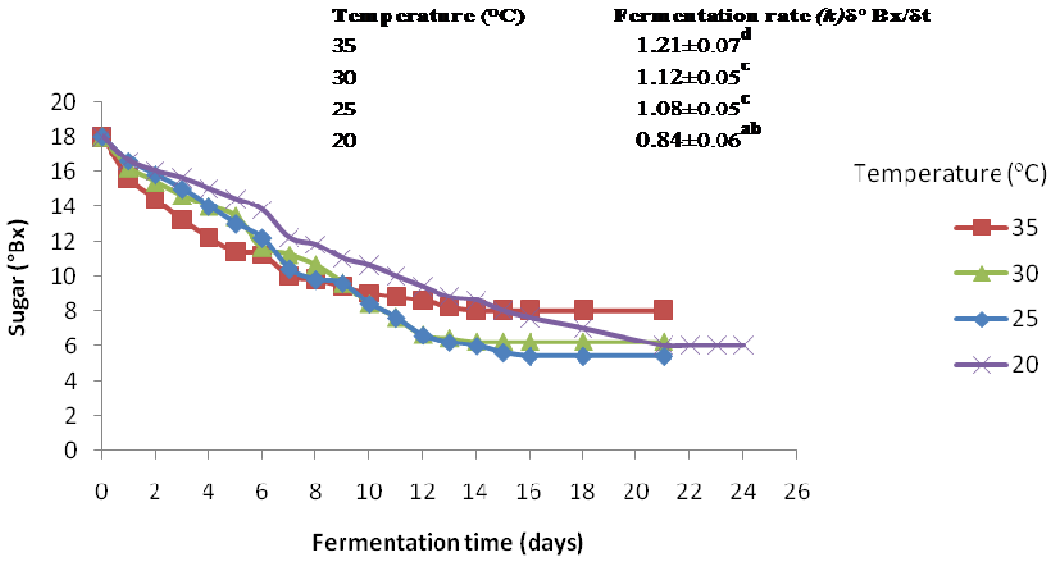

Fig 1: Effects of temperature on substrate utilization and fermentation kinetics at 0.05% yeast inoculum size; Fermentation rate (k) sharing the same superscripts were not significantly different (p>0.05)

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4.3 Effects of yeast inoculum size on the fermentation kinetics of apple mango wine during fermentation: As shown in Fig. 2, the higher the yeast concentration, the higher was the initial fermentation rate. At 0.0065%, innoculation it was slowest and decrease in sugar level was from 18 to 15 ºBx after initial four days of fermentation. At 0.1% yeast concentration, fermentation rate was fastest indicating a decrease in sugar concentration from 18 to 12.4 ºBx after initial four days. Experiments with higher innoculum size, 0.1% and 0.05%, rapidly reached the completion of fermentation as compared to lower innoculum, which could not utilize the sugars completely. Increasing the yeast concentration resulted in a faster fermentation rate with brewer's and wine yeast strains(Mateo and Edelen, 1996, 2001) although these researchers did not mention the specific concentrations of the innoculum sizes. In this study, innoculum size of 0.5%, (results not indicated) the fermentation rate was highest but the sugars could not be completely utilized resulting to wine with very low alcohol content (5.82%). It is therefore important to note that higher innoculum sizes result in higher fermentation rates but at certain levels, the yeast cannot completely utilize the available sugars.

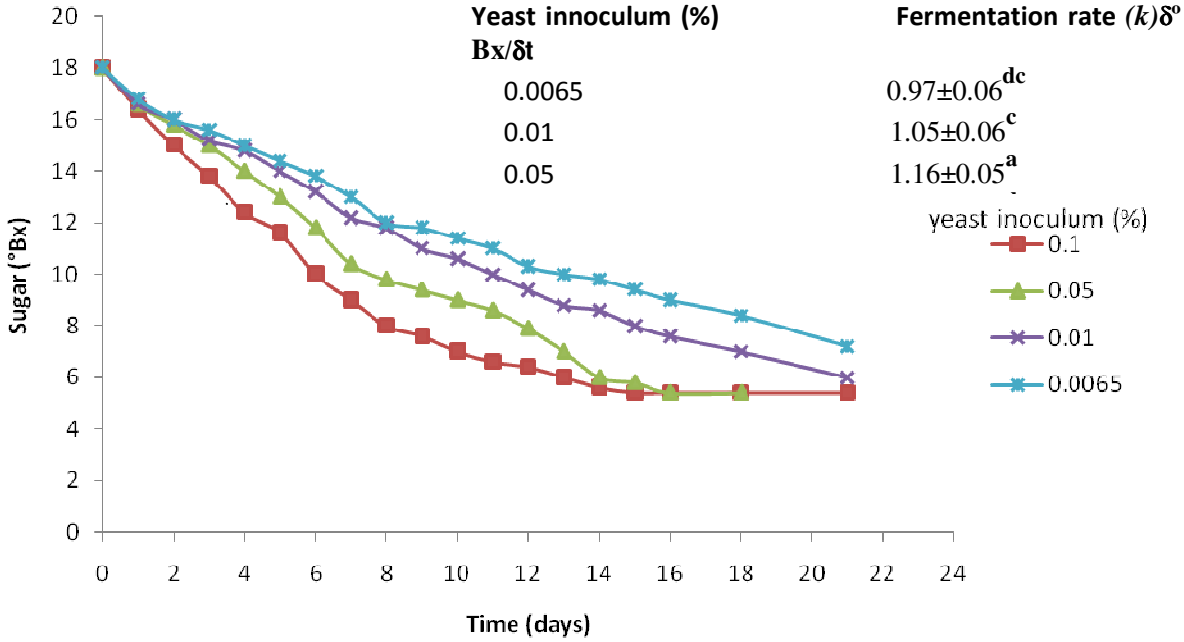

Fig 2: Substrate utilization during the fermentation kinetics of mango juice from Apple variety at 25ºC; The rate of fermentation at varying innoculum sizes is significantly different ($p<0.05$) as shown by the different superscripts

4.4 Effects of temperature on chemical properties of mango wine: Alcohol yield was highest when fermentation was done at 25ºC for all the yeast concentrations. At this temperature, there was maximum conversion of sugars and fermentation took 18 days to completely utilize the available sugars in the mango juice at 0.05 % yeast concentration. Fermentation temperatures of 20°C and 25°C yielded higher alcohol relative to 30°C and 35°C. There was no significant difference ($p>0.05$) on pH and TTA at different temperatures. The concentration of alcohol decreased as the temperature increased, which has been related to a drop in the ethanol yield and a reduced use of substrate (Casey and Ingledew, 1986). This difference in ethanol yield at different temperatures could also be related to biomass production (Ma-Jesu´sTorija, *et al.*, 2003). The content of volatile acids, which measures the degree of sourness of the wine, should be as low as possible (Yannam, *et al.*, 2009). Volatile acidity increased as the temperature increased although the values were within the

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acceptable range of 0.3 to 0.6% reported for wines (Amerine *et al.*, 1980). From this study, low temperatures of 20°C and 25°C yielded low residual sugars, which was attributed to these temperatures as suitable for the proliferation of yeast cells during fermentation.

Table 2: Chemical properties of mango wine at different temperatures at 0.05% *S. cerevisiae* innoculum size and pH of 4.5

Parameters	20°C	25°C	30°C	35°C
Alcohol content (% v/v)	8.07±1.42^{d}	9.44±1.74^{c}	7.20±1.69^{b}	6.93±1.72^{a}
pH	4.01±0.04^{b}	4.01±0.03^{b}	3.99±0.03^{b}	3.99±0.04^{b}
TTA	0.93±0.23^{a}	0.93±0.22^{a}	0.94±0.24^{a}	0.94±0.22^{a}
Residual °Bx	6.0±0.11^{b}	5.4±0.10^{a}	6.0±0.10^{b}	8.0±0.10^{c}
Volatile acidity (% v/v as acetic acid)	0.37±0.17^{a}	0.39±0.17^{a}	0.48±0.18^{d}	0.51±0.17^{b}

Means within the same row with different superscripts were significantly different (p<0.05)
Values are presented as mean ± SD
n=3

4.5 Effects of yeast concentration on chemical properties of mango wine; Table 4 below shows that the level of innoculum size had no effect on pH, titratable acidity, and volatile acidity of the mango wine. Innoculum size of 0.05 % gave the highest alcohol yield as compared to the rest of the innoculum sizes. There was no significant difference (p>0.05) in the physico-chemical properties of mango wine produced from 0.1 % and 0.05 % yeast innoculum sizes except for the alcohol yield. Alcohol production increased with increase in innoculum concentration up to 0.05%. Higher levels of innoculum gave almost same amount of alcohol content, such as 0.05%, innoculation gave 9.44% of alcohol content, while 0.1% innoculum concentration gave 8.67% alcohol. From this, it can be shown that as the concentration of yeast innoculum increased, yeast converted more sugars to alcohol, while at higher concentration yeast was not able to utilize more sugar for conversion as in the case of 0.1%.

Table 4: Chemical properties of mango wine at different inoculum sizes at 25°C

Parameters	0.1%	0.05 %	0.01 %	0.0065% (control)
Alcohol content (% v/v)	8.67±0.04^{d}	9.44±0.04^{c}	7.20±0.04^{b}	6.93±0.04^{a}
pH	4.09±0.02^{b}	4.08±0.02^{b}	4.05±0.02^{b}	4.08±0.02^{b}
TTA	0.93±0.06^{a}	0.93±0.07^{a}	0.94±0.06^{a}	0.93±0.07^{a}
Residual °Bx	5.4±0.10^{a}	5.4±0.10^{a}	6.0±0.20^{b}	7.4±0.10^{c}
Volatile acidity (g/l as acetic acid)	0.37±0.14^{a}	0.37±0.13^{a}	0.38±0.13^{a}	0.37±0.13^{a}

Means within the same row with the same superscript were not significantly different (p>0.05)
Values are presented as mean ± SD
n=3

5.0 CONCLUSION

The increase in fermentation temperature and yeast concentration significantly increased the fermentation kinetics of apple mango wine. However, at high temperature of 35°C and yeast concentration of 0.1% the sugars could not be completely utilized during fermentation yielding low alcohol content. Yeast concentration did not have a significant effect on the chemical properties as

observed with temperature. Fermentation temperature of 25°C and yeast concentration of 0.05% gave the optimal characteristics for the production of apple mango wine using wine yeast. Studies on the influence of yeast concentration on wine quality are limited and therefore further study is still required.

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