



American Journal of
Food Technology

ISSN 1557-4571



Academic
Journals Inc.

www.academicjournals.com

Effects of Processing Pineapple-Based Must into Wines by Anaerobic Fermentation

¹C.O. Ibegbulem, ²P.C. Chikezie, ³C.O. Nweke, ³C.E. Nwanyanwu and ⁴D.C. Belonwu

¹Department of Biochemistry, Federal University of Technology, Owerri, Nigeria

²Department of Biochemistry, Imo State University, Owerri, Nigeria

³Department of Microbiology, Federal University of Technology, Owerri, Nigeria

⁴Department of Biochemistry, University of Port Harcourt, Port Harcourt, Nigeria

*Corresponding Author: P.C. Chikezie, Department of Biochemistry, Imo State University, Owerri, Nigeria
Tel: +2348038935327*

ABSTRACT

Effects of processing pineapple-based must into wines by Anaerobic Fermentation (AnF) only instead of Aerobic and Anaerobic Fermentations (AAnFs) were investigated. Control musts were subjected to aerobic fermentation, AnF and clarification for 7, 83 and 30 days, respectively. Test musts clarified in the course of 90 days AnF. Wines produced by AAnFs were more acidic ($\text{pH}_{\text{test}} = 3.17 \pm 0.01$, $\text{pH}_{\text{control}} = 3.28 \pm 0.01$, $p < 0.05$), had more total acids (test = 0.70 ± 0.01 g tartaric acid/100 mL, control = 0.66 ± 0.00 g tartaric acid/100 mL, $p < 0.05$), fixed acids (test = 0.49 ± 0.02 g malic acid/100 mL, control = 0.39 ± 0.01 g malic acid/100 mL, $p < 0.05$), alcohol (test = $12.72 \pm 0.01\%$, control = $11.36 \pm 0.00\%$, $p < 0.05$). Furthermore, wines produced by AnF had more volatile acids (test = 0.39 ± 0.00 g acetic acid/100 mL, control = 0.33 ± 0.01 g acetic acid/100 mL, $p < 0.05$) and glucose (test = 1.50 ± 0.01 g/100 mL, control = 1.40 ± 0.00 g/100 mL, $p < 0.05$). Pineapple-based must processed into wines by anaerobic fermentation produced organoleptically preferred good quality white dry table pineapple wines with lower derivable energy content.

Key words: Aerobic, anaerobic, fermentation, pineapple, wine

INTRODUCTION

Wine fermentation has two distinct stages: Primary and secondary (also described as aerobic and anaerobic) fermentations (Berry, 1996; Jacobs, 2001). These fermentation stages involve complicated multistep chemical transformation of glucose to ethanol in yeast cells. While the reactions of the aerobic fermentation stage include those of glycolysis, pyruvate dehydrogenase complex, tricarboxylic acid cycle and respiratory (electron transfer) chain leading to conversion of glucose to CO_2 and H_2O , the anaerobic fermentation stage involves glycolysis, pyruvate decarboxylase complex and alcohol dehydrogenase, which converts glucose to ethanol and CO_2 (Garrett and Grisham, 1999; Nelson and Cox, 2000). Primary fermentation is characterized by 'vigorous' chemical reactions in which large volumes of gas are generated, whereas secondary fermentation is a slow and quiet reaction and is barely discernable towards the end (Berry, 1996; Jacobs, 2001). In fermentation practice, the yields of ethanol and CO_2 that vary between 92 and 98% of the theoretical yield are attributable to the formation of small amounts of aldehydes, volatile and fixed acids, glycerol and other connecting substances, utilization of sugar for the yeasts' metabolism and small losses of ethanol during fermentation (Berti, 1981).

Wines also undergo Malo Lactic Fermentation (MLF) (Berry, 1996; De Revel *et al.*, 1999; Jacobs, 2001) leading to reductions in the wine acidity due to conversion of malic acid (a diprotic, dicarboxylic acid) to lactic acid (a monoprotic, monocarboxylic acid) (Berti, 1981; Berry, 1996; Jacobs, 2001; Butz, 2007). MLF promotes stabilization and enrichment of the aromatic compositions of wines associated with aging of wines (De Revel *et al.*, 1999). During aging, the yeast cells die and autolyse, releasing aromatic and flavour some compounds like esters, amino acids and amides (Amerine, 1981; Berti, 1981). A number of yeast species found in wine, e.g., *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *S. pombe van malidevorans* and *Zygosaccharomyces bailii* can also utilize tricarboxylic acid cycle intermediates when grown on glucose (Thornton and Rodriguez, 1996).

Apart from grapes, the use of tropical fruits as substrates for the production of wines has been reported (Berry, 1996; Jacobs, 2001; Okunowo *et al.*, 2005; Reddy and Reddy, 2009; Savage *et al.*, 2013). *Ananascomosus* (pineapple) belongs to the family *Bromeliaceae*. Its varieties include Cayenne, Queen, Spanish and Pernambuco (Macrae *et al.*, 1993; Tatransky, 1997; Bartholomew *et al.*, 2002). Sixty percent of pineapple is edible and contains 80-85% water, 12-15% sugar, 0.6% acid, 0.4% protein, 0.5% ash, 0.1% fat, some fiber and several vitamins (Samson, 1982). Pineapple is largely consumed around the world as canned pineapple slices, chunk and dice, pineapple juice, fruit salads, sugar syrup, alcohol, pineapple chips and pineapple puree (Savage *et al.*, 2013). It is also grown and used as a medicinal plant in the tropics because it contains a proteolytic enzyme called bromelain (Tochi *et al.*, 2008). The therapeutic properties include treatment of malignant cell growth, thrombus formation, inflammation, control of diarrhea, dermatological and skin debridement (Tochi *et al.*, 2008; Savage *et al.*, 2013). A good quality wine has been and can always be produced from pineapple at higher temperatures, as obtained in Nigeria, than recommended (Jacobs, 2001) because pineapple is a tropical plant (Jacobs, 2001; Bartholomew *et al.*, 2002) unlike grape which is normally grown in the temperate regions and whose wines are used as standards (Jacobs, 2001). Wine can be considered a food because of the caloric values of its ethanol, organic acid and sugar contents. However, its alcohol content can cause foetal damage, heart disease, cirrhosis of the liver, oesophageal, breast, colon and pancreatic cancers when abused (Wardlaw and Kessel, 2002; Ibegbulem *et al.*, 2013a).

Most often, musts from which fruit wines are produced are first subjected to Aerobic Fermentation (AF), which can last upto seven days, before subjecting them to anaerobic fermentation (AnF). The AF stage demands the use of extra paraphernalia, space occupied by such paraphernalia, operational time and the attendant risk of contamination when transferring the fermenting must into fermentation jars for AnF. We hypothesized that a good quality wine can also be produced by subjecting the same must to AnF only. The aim of the present study was to compare the properties of pineapple wines produced from must fermented by *S. cerevisiae* using two different fermentation schemes. In one scheme, the must was subjected to the usual AF and AnF schemes, whereas the other scheme involved subjecting the must to the AnF phase only.

MATERIALS AND METHODS

Collection and processing of pineapple fruits: Six large and mature juicy *A. comosus* (Queen Pineapple) fruits and active dried yeast (*S. cerevisiae*) were purchased from Eke-Ukwu Market, Owerri, Nigeria. The fruits were authenticated by Dr. F.N. Mbagwu of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. The reagents used were of

analytical grade and were purchased locally. The fruits were washed with distilled water and the rind peeled off manually. The juicy flesh was cut into slices and juice squeezed out and strained through cheese cloth.

Preparation and separation of pineapple must: The must was prepared as described by Berry (1996). Briefly, 5350.0 mL of filtered juice was fortified by dissolving 4500.0 g of pure granulated sucrose, 30.0 g of citric acid and 10.0 g $(\text{NH}_4)_2\text{SO}_4$. Boiling water (13500.0 mL) was poured in and stirred to dissolve the solutes. An aliquot (200.0 mL) of the must was set aside for analyses. The remaining must was then divided into six volumes of 3210.0 mL each. Three portions were poured into three different 5000.0 mL capacity glass fermentation jars, providing enough ullage ($\approx 790 \text{ cm}^3$) to accommodate froths. The other portions were poured into three different 10000.0 mL capacity plastic buckets with fairly loose lids. They were allowed to cool to $35 \pm 3.0^\circ\text{C}$.

Inoculation and fermentation of musts: The six portions of the must were inoculated with 3.0 g of commercially available active dried baker's yeast (*S. cerevisiae*), respectively. Active Dried Yeast (ADY) is preferred because of its consistency in quality, ease of storage and use (Berti, 1981).

The portions of the must that were placed in the plastic buckets were fermented into wines using the Aerobic and Anaerobic Fermentations (AAnFs) schemes as described by (Berry, 1996; Jacobs, 2001). First, the musts were subjected to AF for 7 days at room temperature ($26.1 \pm 3.0^\circ\text{C}$) by stirring in air vigorously once a day. The vigorous stirring also helped break the scum at the surface of the musts. At the end of the AF phase, the musts were poured into glass fermentation jars and subjected to the AnF phase for 83 days by fitting airlocks onto the mouths of the jars. The wines were then racked (siphoned) off the lees (sediments) into another set of fermentation jars, the airlocks were re-fitted and fermentation and clarification carried out for another 30 days before racking and bottling.

The portions of the must that were poured into the fermentation jars were subjected to the AnF phase only for 90 days as a modification of the method for the production of pineapple wine described by Berry (1996). The modification made was that the AF phase was skipped. Fermentation of portions of the must was also carried out at room temperature ($26.1 \pm 3.0^\circ\text{C}$). The wines clarified in the course of AnF and were racked off the lees and bottled.

Determination of presence of phytochemicals: The presence of some antioxidant phytochemicals like tannins, saponins and flavonoids was detected in triplicates using standard methods (Ayoola *et al.*, 2008).

Measurement of acidity: Concentrations of total acids (tartaric acid g/100 mL), fixed acids (malic acid g/100 mL) and volatile acids (acetic acid g/100 mL) were determined in triplicates using standard methods (Haddad *et al.*, 1978; Butz, 2007). The fixed and volatile acidities were initially calculated as tartaric acids then expressed as malic and acetic acids by multiplying by factors of 1.119 and 1.250, respectively; being the ratios of the equivalent weights of tartaric acid (75.05 g) to malic acid (67.05 g) and tartaric acid to acetic acid (60.05 g), respectively.

Measurement of pH, ethanol and glucose contents: The pH values were determined in triplicates with the aid of a digital pH meter (Labtech, India) (AOAC, 2006). The presence of ethanol was detected using Jones Reagent as described by Ibegbulem (2012). Ethanol contents

were determined by the specific gravity method (Haddad *et al.*, 1978; AOAC, 2006; Ibegbulem *et al.*, 2013b). Glucose concentrations were determined using the methods of Plummer (1971). Ascorbic acid concentrations were determined by an established method (AOAC, 2006).

Derivable energy content: Derivable energy contents were calculated in triplicates by multiplying their fixed acid, volatile acid, ethanol and glucose contents by factors 3, 3, 7 and 4, respectively. The derivable energy g⁻¹ of organic acids, ethanol and glucose are 3, 7 and 4 kcal, respectively (Codex Alimentarius, 2001; Wardlaw and Kessel, 2002; FAO, 2003).

Measurement of mineral content: Mineral (Ca, Cu, Mg, Fe, Mn and Zn) contents were determined in triplicates using an atomic absorption spectrophotometer (product of Perkin Elmer, USA) as described by Allen *et al.* (1996) and AOAC (2006).

Microbial analyses: Microbial analyses were carried out in triplicates by both microscopic inspection and plating of samples of the wines on dextrose and nutrient agar plates (Taylor *et al.*, 1998). Plating of the samples were repeated using agar plates containing 100 mg L⁻¹ cycloheximide.

Sensory evaluation: Sensory evaluation was performed by ten well-trained panelists using a 5 point hedonic scale where, 0 = Unacceptable, 1 = Poor, 2 = Satisfactory, 3 = Good, 4 = Very good and 5 = Excellent. Threshold score of each attribute was used for the interpretation.

Reaction rates: The reaction rate for a parameter was calculated as the ratio of the difference between the values of that parameter in the must and in the wine to the total time spent. For the wines produced by the AAnFs, time was 172800 min (for instance, 120 days because of the assumption that there were biochemical changes during the clarification stage). Wines that were produced by the AnF scheme only, had fermentation times of 129600 min (for instance, 90 days since the wines stopped fermenting and clarified at that point).

Statistical analyses: Wines were produced in triplicates and analyses for a parameter in each wine carried out in triplicates resulting in 9 determinations. Parameters in the must were estimated 9 times. Data were analyzed using the one-way analysis of variance (ANOVA) and student's t test (Field, 2005). Mean was considered significantly different at p<0.05.

RESULTS AND DISCUSSION

The study technically represents the case of employing different fermentation schemes to process the same substrate into the same product. One scheme involved the reactions of functional mitochondria while the other involved dysfunctional mitochondria yet faced with some intermediates of functional ones.

The phytochemicals detected in the musts and wines were saponins, tannins and flavonoids. We did not quantify them because they are plant secondary products (Wardlaw and Kessel, 2002) and are known not to be substrates for fermentation. These phytochemicals must have originated from the pineapple juice used. They are normal constituents of wines

Table 1: Acidities, fixed acidity/volatile acidity ratio, pH, alcohol, glucose, ascorbic acid and derivable energy contents of the must and wines†

Parameters	Must	Wine production process	
		AAnFs	AnF
Total acidity (TA, tartaric acid g/100 mL)	0.82±0.02 ^a	0.70±0.01 ^b	0.66±0.00 ^c
Fixed acidity (FA, malic acid g/100 mL)	0.78±0.00 ^a	0.49±0.02 ^b	0.39±0.01 ^c
Volatile acidity (VA, acetic acid g/100 mL)	0.15±0.01 ^a	0.33±0.01 ^b	0.39±0.00 ^c
FA/VA ratio	5.20±0.00 ^a	1.64±0.61 ^b	1.10±0.91 ^c
pH	4.05±0.00 ^a	3.17±0.01 ^b	3.28±0.01 ^c
Ethanol (% v/v)	0.00±0.00	12.72±0.01 ^b	11.36±0.00 ^c
Glucose (g/100 mL)	33.92±0.05 ^a	1.40±0.00 ^b	1.50±0.01 ^c
Ascorbic acid (mg/100 mL)	4.68±0.01 ^a	4.68±0.01 ^b	4.68±0.00 ^c
Derivable energy content (kcal/100 mL)	138.47±0.12 ^a	97.10±0.07 ^w	87.88±0.05 ^a

AAnFs: Aerobic and anaerobic fermentations, AnF: Anaerobic fermentation, Values on the same row bearing the same superscript letter are not significantly different ($p > 0.05$), †: Results are Mean±SD of 9 determinations

(Hennig and Burkhardt, 1960; Wardlaw and Kessel, 2002; Basha *et al.*, 2004; Puskas and Miljic, 2012) and act as antioxidants, protecting wines and their consumers from free radical and oxidative damage (Wardlaw and Kessel, 2002).

The must contained significantly ($p < 0.05$) lower amounts of volatile acids (Table 1). However, total acids, fixed acids and fixed acidity/volatile acidity ratio were significantly ($p < 0.05$) higher than those in the wines. Lower concentrations of total acids in wines relative to those in must have been reported (Butz, 2007); contrasting with the report of Thoukis *et al.* (1965) which stated that total acid levels increased in fermented medium during alcoholic fermentation by yeast. There were also reductions in the concentrations of fixed acids in the wines relative to the must. While it can be argued that yeast cells synthesize organic acids and leech them into the fermented medium, mostly during AF, yeasts also multiply and use up much of these organic acids as sources of energy and biosynthetic intermediates such as in the synthesis of valine. The total and fixed acids in the wines that were produced only by the AnF scheme (AnF wines) were lower than those in the wines that were produced sequentially by AAnFs (AAnFs wines). These may have been due to the metabolism of more of the must's fixed acids. Malic acid, being a fixed acid, can be fermented to lactic acid, acetic acid and alcohol through MLF, Malo Pyruvic Acetic Acid Fermentation (MPAAF) and Malo Pyruvic Ethanolic Fermentation (MPEF) pathways, respectively (Thoukis *et al.*, 1965; Saayman and Viljoen-Bloom, 2006; Butz, 2007) or used to produce intermediates of biosynthesis. These pathways involve the initial decarboxylation of malic acid by an intracellular malic enzyme to pyruvic acid. Malic acid can also be dehydrated by fumarase to fumaric acid (Garrett and Grisham, 1999; Nelson and Cox, 2000). MPEF is mostly carried out by yeast species such as *S. pombe* and strains of *S. cerevisiae* (Volschenk *et al.*, 2003). The reductions in the concentrations of fixed acids and increase in those of volatile acids in the wines relative to those in the must (Table 1) suggested that the malic acid may have been fermented by the MPAAF and/or MLF pathways. Utilization of these pathways seemed to have been higher during the AnF phase, as was noticed in the AnF wines. The changes in the concentrations of the acidities supported the report of Thoukis *et al.* (1965) which stated that there are changes in the organic acid compositions of a fermented medium during alcoholic fermentation by yeast. The concentrations of the total acids in the wines fell within the recommended range of 0.5-1.0% (Amerine *et al.*, 1979; Pandell, 1999).

The concentrations of their volatile acids were however higher than the recommended level (<0.3 g/100 mL) as reported by Saayman and Viljoen-Bloom (2006) between 3.0-10.0%. The variations in the wines' acidities resulted in a 38.20% difference in their fixed acidity/volatile acidity ratios suggesting that the AAnFs wines could have the same margin of longer shelf-life than the AnF wines.

The pineapple wines produced by the two different fermentation schemes had significantly ($p < 0.05$) lower pH values and glucose contents compared to those of the must. However, the alcohol contents of the AAnFs wines were significantly ($p < 0.05$) higher than those of the AnF wines. The must did not contain ethanol and ascorbic acid content was not affected by the fermentation schemes, suggesting that ascorbic acid was neither degraded nor synthesized during the fermentation processes.

The lower concentrations of total acids in the AnF wines (Table 1) were responsible for their lower acidity (higher pH value). Their lower alcohol contents also buttressed the suggestion that malic acid fermentation process undertook more of the MPAAF and/or MLF pathway than the MPEF pathway. If they had undertaken the MPEF pathway, they would have contained more ethanol than they did. Alcohol is also produced during AF but a significant portion of the yeast's energy is rather devoted to reproductive events (Kraus, 2012). Some of the malic acid in the must and wines originated from the pineapples according to previous reports of Singh (2013). The lower sugar contents of the AAnFs wines suggested that substantial quantity of the sugar was fermented during the AF phase. The relatively higher alcohol and lower glucose contents also suggested that the AF phase may have produced more yeast cells, which used up more glucose and produced more ethanol. Pineapple wine production by the AnF or AAnFs significantly ($p < 0.05$) reduced the derivable energy contents of the must (Table 1) between 29.88-36.53%. Much of the energy value was lost due to the fermentation of the glucose contents. These were indications that fermentation as a food-processing method is an energy-content depleting process. Glucose contributed 135.68 kcal/100 mL of the derivable energy value of the must, whereas the ethanol contents of the wines were major contributors to derivable energy contents, 89.04 kcal/100 mL for AAnFs wines and 79.54 kcal/100 mL for AnF wines. Although, the protein contents of the must and wines were not measured as a function of energy derivable from nitrogenous compounds, the importance of the energy values of the wines' stable protein contents is acknowledged and suggested for future studies. However, previous reports had stated that energy yield g^{-1} protein is approximately 4 kcal (Codex Alimentarius, 2001; Wardlaw and Kessel, 2002; FAO, 2003). The derivable energy contents of the wines (Table 1) seemed to suggest that a cup (250 mL capacity) of the AAnFs wines can sustain a sedentary individual for 161.83 min, whereas the same volume of AnF wines can sustain the same individual for 146.48 min.

Pineapple wine production by the AAnFs reduced wine mineral contents (Table 2). This may be attributed to precipitation of wine lees, much of which are deposited as calcium and potassium tartarates (Berry, 1996; Jacobs, 2001; Butz, 2007). It has been reported that yeast population is much higher during AF than during AnF, being multiplied between 100-200 times during AF (Berry, 1996; Jacobs, 2001; Kraus, 2012) and expected to grow 5-10 folds during AnF (Berti, 1981). In this study, the AAnFs wines deposited more lees and this may have been responsible for the loss of some minerals. Pineapple wine lees have been reported to contain relatively high levels of crude protein, ether extract, soluble carbohydrates, crude fiber, dead yeast cells and ash (Anyaehe and Nkwocha, 2003).

Table 2: Mineral contents of the wines†

Minerals (mg L ⁻¹)	Wine production process	
	AAnFs	AnF
Calcium	43.00±0.02	52.00±0.03*
Copper	16.00±0.00	24.00±0.05*
Magnesium	8.00±0.01	10.00±0.01*
Iron	22.00±0.03	29.00±0.01*
Manganese	8.00±0.00*	6.00±0.03
Zinc	20.00±0.01	26.00±0.01*

AAnFs: Aerobic and anaerobic fermentations, AnF: Anaerobic fermentation, *Significantly (p<0.05) higher, †Results are Mean±SD of 9 determinations

Table 3: Sensory properties of the wines

Attributes	Wine production process			
	AAnFs		AnF	
	Score†	Interpretation	Score†	Interpretation
Aroma	3.00±0.01	Good	3.60±0.02*	Good
Taste	3.30±0.01	Good	3.40±0.00*	Good
Mouthfeel	3.30±0.02	Good	4.00±0.01*	Very good
Colour	4.00±0.01	Very good	4.30±0.00*	Very good
Clarity	4.20±0.01	Very good	4.50±0.01*	Very good
Overall acceptability	3.90±0.01	Good	4.00±0.01*	Very good

AAnFs: Aerobic and anaerobic fermentations, AnF: Anaerobic fermentation, *Significantly (p<0.05) higher, †Values are Mean±SD of 10 respondents

There was no microbial growth on cultured wine samples. This suggested that the wines did not contain living or wild yeast cells or any contaminating microorganisms thereby making the wines safe for consumption. The cycloheximide used during the assay inhibited yeast growth, whereas the growth of wild type yeast was not affected (Pandell, 1999).

The mean scores for the sensory properties of the AnF wines were significantly (p<0.05) higher than those of the AAnFs wines (Table 3). The mouthfeel of AnF wines and overall acceptability attributes were preferred to the AAnFs wines. The lower total acidity, alcohol and higher glucose and pH values of AnF wines (Table 1) may have been responsible for the preference. These parameters affect the tastes (tartness and sourness) and aroma of wines (Pandell, 1999). Total acidity is a major factor that affects taste (Butz, 2007). Tartness of sourness is a sensory perception of hydrogen ions on the taste buds (Nelson and Cox, 2000; Butz, 2007). The increased concentrations of volatile acids, like lactic acid (Thoukiss *et al.*, 1965), may have enriched their aromatic compositions (De Revel *et al.*, 1999). The nature of organic acid and ethanol contents of AnF wines contributed to the characteristic sweet smelling esters aroma (Hill and Holman, 1986; Butz, 2007) and the relatively high glucose content resulted to sweeter wine (Berry, 1996; Jacobs, 2001; Butz, 2007). The organic acids in the AAnFs wines also enriched the aromatic compositions.

The rates of change in the levels of parameters proceeded faster (p<0.05) during AnF (Table 4) contrary to earlier reports of (Berry, 1996; Jacobs, 2001). The present findings showed

Table 4: Rate of change of some parameters in the wines†

Parameters	Wine production process	
	AAnFs	AnF
Δ TA (g dL ⁻¹ min ⁻¹)	6.88×10 ⁻⁷ ±6.20×10 ⁻⁸	1.24×10 ⁻⁶ ±1.55×10 ⁻⁷ *
Δ FA (g dL ⁻¹ min ⁻¹)	1.67×10 ⁻⁶ ±1.10×10 ⁻⁷	3.01×10 ⁻⁶ ±8.00×10 ⁻⁸ *
Δ VA (g dL ⁻¹ min ⁻¹)	1.04×10 ⁻⁶ ±0.00×10 ⁰	1.85×10 ⁻⁶ ±8.00×10 ⁻⁸ *
Δ pH (min ⁻¹)	5.09×10 ⁻⁶ ±6.00×10 ⁻⁸	5.94×10 ⁻⁶ ±8.00×10 ⁻⁸ *
Δ Alcohol (% min ⁻¹)	7.37×10 ⁻⁵ ±5.00×10 ⁻⁸	8.76×10 ⁻⁵ ±0.00×10 ⁰ *
Δ Glucose (g dL ⁻¹ min ⁻¹)	1.88×10 ⁻⁴ ±2.90×10 ⁻⁷	2.50×10 ⁻⁴ ±3.06×10 ⁻⁷ *
Δ Ascorbic acid (mg dL ⁻¹ min ⁻¹)	0.00×10 ⁰ ±0.00×10 ⁰	0.00×10 ⁰ ±0.00×10 ⁰

AAnFs: Aerobic and anaerobic fermentations, AnF: Anaerobic fermentation, Δ: Change in concentration, TA: Total acidity, FA: Fixed acidity, VA: Volatile acidity, *Significantly (p<0.05) higher, †Based on Table 1, Values are Mean±SD of 9 determinations

that both rate and total amount of glucose consumption were many times greater under AnF than AF because of low adenosine triphosphate (ATP) yield (Nelson and Cox, 2000). ATP is a regulator of aerobic and anaerobic fermentation (Garrett and Grisham, 1999; Nelson and Cox, 2000). Though, the concentration of glucose was higher in the AnF wines (Table 1), its rate of consumption by the yeast cells was comparatively higher than AAnFs wines (Table 4).

CONCLUSION

Production of good quality pineapple wines by AnF practically reduced the cost of production by eliminating the need for the paraphernalia used during AF. It reduced our operational time by 43200 min since the AnF-processed wines had clarified during AnF. Wine production by this scheme produced organoleptically preferred good quality white dry table pineapple wines with lower derivable energy content.

REFERENCES

- AOAC, 2006. Official Methods of Analysis of the AOAC. 18th Edn., Association of Official Analytical Chemists, Washington, DC., USA.
- Allen, S.E., J.A. Parinson and C. Quarmry, 1996. Chemical Analysis of Ecological Materials. Blackwell, Oxford.
- Amerine, M.A., 1981. Wine Production Technology in the United States. Vol. 145, American Chemical Society, USA., ISBN: 9780841205963, pp: 1-27.
- Amerine, M.A., H.W. Berge, R.E. Kunkee, C.S. Ough, V.L. Singleton and A.C. Webb, 1979. The Technology of Wine Making. 4th Edn., AVI Publishing Co., Westport, USA.
- Anyaehe, A.A. and G.A. Nkwocha, 2003. Replacement value of pineapple wine sediment for maize offal in grower/finisher pigs diet. J. Agric. Food Sci., 1: 89-95.
- Ayoola, G.A., S.S. Ipav, M.O. Sofidiya, A. Aderonke, A. Bello, H.A.B. Coker and T.O. Odugbemi, 2008. Phytochemical screening and free radical scavenging activities of the fruits and leaves of *Allanblackia floribunda* Oliv. (Guttiferae). Int. J. Health Res., 1: 87-93.
- Bartholomew, D.P., R.E. Paul and K.G. Rohrbach, 2002. The Pineapple: Botany, Production and Uses. CABI Publishing, Wallingford, UK., ISBN: 9780851999791, Pages: 320.
- Basha, S.M., M. Musingo and V.S. Colova, 2004. Compositional differences in the phenolics compounds of muscadine and bunch grape wines. Afr. J. Biotechnol., 3: 523-528.

- Berry, C.J.J., 1996. First Step in Wine Making. Nexus Special Interests Ltd., Hertfordshire, UK.
- Berti, L.A., 1981. Sparkling Wine Production in California. In: Wine Production Technology in the United States (ACS Symposium Series, Vol. 145), Amerine, M.A. (Ed.). American Chemical Society, USA., pp: 85-121.
- Butz, E., 2007. Practical considerations for managing wine acidity. Proceedings of the Spring Workshop of Purdue Wine Grape Team, March 26, 2007, Ertel Cellars, Batesville.
- Codex Alimentarius, 2001. Codex guidelines on nutrition labeling CAC/GL 2-1985. CODEX Alimentarius, International Food Standard, USA.
- De Revel, G., N. Martin, L. Pripis-Nicolau, A. Lonvaud-Funel and A. Bertrand, 1999. Contribution to the knowledge of malolactic fermentation influence on wine aroma. *J. Agric. Food Chem.*, 47: 4003-4008.
- FAO, 2003. Food energy-methods of analysis and conversion factors. Food and Nutrition Paper 77, Food and Agriculture Organization of the United Nations, Rome, Italy, pp: 1-93.
- Field, A.P., 2005. Discovering Statistics Using SPSS. 2nd Edn., SAGE Publication Ltd., London.
- Garrett, R.H. and C.M. Grisham, 1999. Biochemistry. 2nd Edn., Brooks/ Cole, Pacific Groove, CA., USA.
- Haddad, P.R., M. Sterns and J. Wardlaw, 1978. Analysis of wine: An undergraduate project. *Edu. Chem.*, 15: 87-89.
- Hennig, K. and R. Burkhardt, 1960. Detection of phenolic compounds and hydroxy acids in grapes, wines and similar beverages. *Am. J. Enol. Viticult.*, 11: 64-79.
- Hill, G.C. and J.S. Holman, 1986. Chemistry in Context. 2nd Edn., Thomas Nelson and Sons Ltd., London.
- Ibegbulem, C.O., 2012. Biochemical implications of preserving *Raphiahookeri* palm wine by heating. *J. Res. Biochem.*, 1: 042-046.
- Ibegbulem, C.O., C.U. Igwe, G.N. Okwu, C.O. Ujowundu, E.N. Onyeike and E.O. Ayalogu, 2013a. Total amino acid profiles of heat-processed fresh *Elaeis guineensis* and *Raphia hookeri* wines. *Food Chem.*, 138: 1616-1620.
- Ibegbulem, C.O., E.U. Eyong and E.U. Essien, 2013b. Nutritional and toxicological implications of drinking heat-treated fresh *Raphiahookerisap* in rats. *Nig. J. Biochem. Mol. Biol.*, 28: 1-10.
- Jacobs, F., 2001. Making Wine from Pineapple. Ithem Davis Press Ltd., Owerri.
- Kraus, E.C., 2012. Wine fermentation 101. <http://www.eckraus.com/wine-making-101/>.
- Macrae, R., R.K. Robinson and M.J. Sadler, 1993. Wine. In: Encyclopedia of Food Science Food Technology and Nutrition, Macrae, R., R.K. Robinson and M.J. Saddler (Eds.). Harcourt Brace Jovanovich Publishers, New York, pp: 4921-4946.
- Nelson, D.C. and M.M. Cox, 2000. Lehninger Principles of Biochemistry. 3rd Edn., Worth Publishers, New York, USA., Pages: 842.
- Okunowo, W.O., R.O. Okotore and A.A. Osuntoki, 2005. The alcoholic fermentative efficiency of indigenous yeast strains of different origin on orange juice. *Afr. J. Biotechnol.*, 4: 1290-1296.
- Pandell, A.J., 1999. The acidity of wine. http://www.wineperspective.com/the_acidity_of_wine.htm.
- Plummer, D.T., 1971. An Introduction to Practical Biochemistry. McGraw-Hill, London, UK.
- Puskas, V.S. and U.D. Miljic, 2012. Effects of fining on phenolic compounds and colour of red wine obtained with addition of increased amounts of grape solid phase in pomace. *Hem. Ind.*, 66: 727-734.
- Reddy, L.V.A. and O.V.S. Reddy, 2009. Effect of enzymatic maceration on synthesis of higher alcohols during mango wine fermentation. *J. Food Qual.*, 32: 34-47.

- Saayman, M. and M. Viljoen-Bloom, 2006. The biochemistry of malic acid metabolism by wine yeasts: A review. *S. Afr. J. Enol. Viticult.*, 27: 113-122.
- Samson, J.A., 1982. *Tropical Fruits*. 2nd Edn., Longman Publishers, New York.
- Savage, G., O. Tuncay, S. Mason and L. Vanhanen, 2013. Pineapple. <http://foodscience.wikispaces.com/Pineapple>.
- Singh, K., 2013. Malic acid food sources and health benefits. March 6, 2013, <http://loyfly.com/malic-acid-food-sources-and-health-benefits/>
- Tatransky, V., 1997. Pineapple. In: *Academic American Encyclopedia*, Grolier Incorporated (Ed.). Grolier Inc., USA., pp: 320-322.
- Taylor, D.J., N.P.O. Green and G.W. Stout, 1998. Microbiology and Biotechnology. In: *Biological Science*, Soper, R. (Ed.). 3rd Edn., Cambridge University Press, Cambridge, pp: 375-416.
- Thornton, R.J. and S.B. Rodriguez, 1996. Deacidification of red and white wines by a mutant of *Schizosaccharomyces malidevorans* under commercial winemaking conditions. *Food Microbiol.*, 13: 475-482.
- Thoukis, G., M. Ueda and D. Wright, 1965. The formation of succinic acid during alcoholic fermentation. *Am. J. Enol. Viticult.*, 16: 1-8.
- Tochi, B.N., Z. Wang, S.Y. Xu and W. Zhang, 2008. Therapeutic application of pineapple protease (bromelain): A review. *Pak. J. Nutr.*, 7: 513-520.
- Volschenk, H., H.J.J. van Vuuren and M. Viljoen-Bloom, 2003. Malo-ethanolic fermentation in *Saccharomyces* and *Schizosaccharomyces*. *Curr. Gen.*, 43: 379-391.
- Wardlaw, G.M. and M.W. Kessel, 2002. *Perspective in Nutrition*. 5th Edn., McGraw-Hill, Boston.