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Studies on Qualitative and Quantitative Characterization of Alcoholic Beverages from Tropical Fruits

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Abstract: Fermentation of the tropical fruits which involved the activities microorganisms resulted to the alcohol yielded, aroma, taste and the overall acceptability of the products. Six different alcoholic beverages from watermelon, watermelon-banana and watermelon-pineapple mixtures were produced using monoculture and mixed culture fermentation techniques. Three yeast species (*Kleochera apiculata*, *Torulospora delbruckii*, *Saccharomyces cerevisiae*) and six bacteria species (*Aerobacter aerogenes*, *Chromobacterium violacium*, *Lactobacillus* sp., *Leuconostoc oenos*, *Micrococcus luteus* and *Streptococcus lactis* were identified during the study. The daily succession of these organisms in the various fermenting samples, differs in cell mass and occurrence due to their different growth conditions and factors present. A higher bacterial load (4.4±0.3-4.9±0.4 log (cfu) mL^{-1}) than yeast (3.0±00-4.9±0.2 log (cfu) mL^{-1}) counts was observed in the mixed culture fermentation, while in the monoculture fermentation, a higher yeast load (4.1±0.2-4.9±0.3 log (cfu) mL^{-1}) than bacterial loads (2.5±0.1-4.3±0.3 log (cfu) mL^{-1}) counts was recovered. The monoculture fermented beverages were of better characteristics than the mixed culture fermented alcoholic beverages.

Key words: Microbiological, quality, beverages, tropical, fruits, fermentation

INTRODUCTION

Wine is any alcoholic beverage produced from juices of variety of fruits by fermentative action of microorganisms either spontaneously or seeding with a particular strain mainly of yeast species to adopt a particular quality of wine. Ideally, distinctive flavours of wine originate from raw materials during alcoholic and malolactic fermentation (Cole and Noble, 1995). All over the world, different raw materials are used for the production of alcoholic beverages according to tradition. The forms of alcoholic beverage consumed in parts of the world vary considerably according to location and ingredients (Allan, 1983; Block and Glen, 2000).

Microorganisms are fundamental to wine making industries. In addition to microbial succession, another index of interest is the quality attribute (Jay, 1996; Ward and Baj, 1988; Sanni, 1985) isolated the following microorganism from ripened plantain fermented alcoholic beverage (agadagidi): *Saccharomyces cerevisiae*, *Saccharomyces chevaleri*, *Bacillus subtilis*, *Lactobacillus mesenteriods*, *Streptococcus lactis* and *Micrococcus* sp.

There has been some controversy over the relative merits of spontaneous fermentations with natural flora of the 'must' and fermentation carried out with selected yeast strains. While Benda (1982) found that spontaneous fermentation produced a better rounded and more complex aromatic quality, Smhitte *et al.* (1984) found it a significant preference for wine produced with selected yeast.

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Seeding of the fermentation is undertaken with the assumption and expectation that the inoculated strain will out-compete and dominate over indigenous strains of *Saccharomyces cerevisiae* and the non-saccharomyces yeasts. Although there is high probability that inoculated *S. cerevisiae* will dominate the fermentation, seeding will not necessarily guarantee the dominance of any particular strain or its exclusive contribution in the fermentation (Shuts and Gather, 1993; Querol *et al*., 1999).

This study is expected to provide information on microbial population, succession in alcoholic beverage produced from some tropical fruits by mixed and monoculture fermentation. It will also provide information on the beverages qualities base on fermentation strategy and fruit mixtures.

MATERIALS AND METHODS

Sources of Materials

Watermelon (*Citrullus lunatus* Thumb) banana (*Musa sapientum*) and pineapple (*Ananas comosus*) were purchased in Akure metropolis in Ondo State, Nigeria, in 2007.

Materials Preparation

All glass wares were washed with detergent, rinsed severally in clean water, dried and were sterilized in hot air oven at 160°C for 2½ h. The plastic containers, stainless steel trays, kitchen knife and the warring blender used in the research were sterilized in a cabinet with UV light while the work bench was swabbed with cottonwool soaked in absolute ethanol. Pineapple and watermelon, were washed, peeled and chopped aseptically with sterile knife wile banana fruits were aseptically hand peeled without fingers touching the flesh.

Preparation of Musts

The first treatment set up involved a homogenate of 8000 g of watermelon. The 2nd treatment contained 8000 g each of homogenized watermelon and banana while the 3rd treatment was a homogenate of 8000 g each of watermelon and pineapple. However, each of the treatments was replicated for (monoculture and mixed culture fermentations.

Fermentation Process

The fermentation was carried out at room temperature of 28±2°C. In the mixed culture fermentation, indigenous microflora of the fruits were allowed for the fermentation while in the monoculture, the substrates were seeded with brewer's yeast of quantity 8.62 log (cfu) m^{-1} to overgrow the indigenous microflora of the fruits. All the treatments were allowed to ferment for 5 days.

Determination of Alcohol (%)

At every 24 h, alcohol content was determined in the musts using alcohol meter. This was simply measured by immersing the alcohol meter into 20 mL of the must samples. The percentage alcohol was read off from the meter.

Isolation and Enumeration of Microorganisms

At every 24 h, samples were aseptically withdrawn from the fermentors, serially diluted and 1 mL each pure laced in triplicates on nutrient agar and incubated at 30°C for 24 h (bacterial growth) and Potato Dextrose Agar (PDA) incubated at 28°C for 72 h (molds and yeast) growth in accordance with Madigan *et al*. (1997). Resultant colonies were enumerated with Gallenkamp colony counter, purified by streaking method on freshly prepared Nutrient agar and Potato dextrose agar; characterized and identified with the criteria of Holt *et al*. (1994) and yeasts were identified based on the criteria of Kreger Van Rij (1984).

Sensory Evaluation

The filtered beverage samples were tested for sensory evaluation using the multiple comparison test (Ihekoroye and Ngody, 1985). The sensory parameters evaluated are taste, colour and aroma were assessed for overall acceptability. The filtered beverage samples were served chilled in white glass cups in an open space under a bright daylight. With a 10-member panel of regular local beverage consumers, three glass cups each from each replicate were served. The parameters were rated on a 9-point hedonic scale. The ratings were described as dislike extremely (1), dislike very much (2), no preference (5), like extremely (6), like moderately (7), like very much (8) and like extremely (9).

The data obtained were analysed using the analysis of variance (ANOVA) to determine differences and Duncan's Multiple Range Test (DMRT) to separate the means (Duncan, 1955).

RESULTS

Nine different microorganisms were identified. Six were bacteria species and includes: *Micrococcus luteus*, *Leuconostoc oenos*, *Aerobacter aerogenes*, *Chromobacterium violacium*, *Lactobacillus* sp. and *Streptococcus lactis* while three species of yeast were also identified and are *Kleochera apiculata*, *Torulospora delbruckii* and *Saccharomyces cerevisiae*. Among these organisms, only *Saccharomyces cerevisiae* successfully occurred throughout the days of fermentation in both the monoculture and mixed cultures. *Leuconostoc oenos* and *Lactobacillus* sp. were not identified in the fermenting musts at the early stages of fermentation but were prominent toward the end of the fermentation duration. On the other hand, *K. apiculata* and *T. delbruckii* were present in the first two days of fermentation and could not be isolated from the must thereafter.

S. cerevisiae developed in the fermenting musts in an increasing trend along days of fermentation. Though substantial yeast counts was recorded in the mixed culture fermentation, it was found higher in the monoculture fermentation.

Viable Microbial Count During Fermentation

Generally, the mixed culture fermented samples had more bacterial population than yeast (Table 1). In the watermelon fermenting medium, bacterial counts between 4.8±0.1-4.9±0.4 log (cfu) mL^{-1} was observed, in watermelon-banana mixture medium, a lower yeast and higher bacterial counts was however recorded and was between 4.8±0.3-4.9±0.4 log (cfu) mL^{-1} and 3.9-4.3±0.2 log (cfu) mL^{-1}, respectively. The microbial count in watermelon-pineapple mixture was the least, having bacterial counts between 4.4±0.3-4.7±0.1 log (cfu) mL^{-1} and yeast counts of between 3.0-4.3±0.1 log (cfu) mL^{-1} (Table 1, 2).

Table 1: Changes in bacterial counts (log cfu mL^{-1}±SD) during mixed culture fermentation

Day	Watermelon	Watermelon+banana	Watermelon+pineapple
0	4.8±0.1	4.8±0.3	4.4±0.3
1	4.8±0.3	4.8±0.3	4.5±0.4
2	4.8±0.3	4.8±0.4	4.6±0.1
3	4.7±0.4	4.8±0.4	4.5±0.4
4	4.8±0.2	4.9±0.2	4.6±0.3
5	4.9±0.3	4.9±0.4	4.7±0.1

Values are means±SD for three readings

Table 2: Changes in yeast counts (log cfu mL^{-1}±SD) during mixed culture fermentation

Day	Watermelon	Watermelon+banana	Watermelon+pineapple
0	4.6±0.3	3.9±0.0	3.0±0.0
1	4.6±0.0	3.9±0.2	3.7±0.3
2	4.8±0.1	3.8±0.1	3.7±0.3
3	4.8±0.1	3.8±0.1	4.2±0.1
4	4.8±0.2	3.9±0.0	4.3±0.0
5	4.9±0.2	4.3±0.2	4.3±0.1

Values are means±SD for three readings

Table 3: Changes in bacterial counts (log cfu mL^{-1}±SD) during monoculture fermentation

Day	Watermelon	Watermelon+banana	Watermelon+pineapple
0	3.0±0.0	2.6±0.0	2.5±0.1
1	3.9±0.0	2.8±0.1	2.6±0.0
2	3.9±0.3	3.9±0.0	3.5±0.1
3	4.1±0.1	4.0±0.1	3.9±0.4
4	4.2±0.1	4.3±0.0	4.2±0.1
5	4.3±0.3	4.3±0.3	4.3±0.2

Values are means±SD for three readings

Table 4: Changes in yeast counts (log cfu mL^{-1}±SD) during monoculture fermentation

Day	Watermelon	Watermelon+banana	Watermelon+pineapple
0	4.6±0.1	4.6±0.0	4.6±0.0
1	4.1±0.2	4.7±0.0	4.6±0.0
2	4.6±0.1	4.8±0.1	4.6±0.0
3	4.6±0.0	4.8±0.1	4.6±0.0
4	4.9±0.1	4.8±0.0	4.7±0.1
5	4.9±0.3	4.9±0.1	4.7±0.1

Values are means±SD for three readings

Table 5: Changes in alcohol content (%) at mixed culture fermentation of fruits

	Hour of fermentation					
Samples	0	24	48	72	96	120
Wmm	-	-	0.40	0.56	0.70	1.12
WmBm	-	-	2.40	2.90	4.00	4.08
WmPm	-	-	2.80	3.40	4.60	5.00

Wmm = Watermelon mixed culture fermentation, WmBm = Watermelon Banana mixed culture fermentation, WmPm = Watermelon Pineapple mixed culture fermentation

Table 6: Changes in alcohol content (%) at mixed culture fermentation of fruits

	Hour of fermentation					
Samples	0	24	48	72	96	120
Wmm	-	-	1.20	1.80	2.00	2.60
WmBm	-	0.65	1.10	2.00	4.30	5.11
WmPm	-	0.10	1.15	3.05	5.03	6.26

Wmm = Watermelon mixed culture fermentation, WmBm = Watermelon Banana mixed culture fermentation, WmPm = Watermelon Pineapple mixed culture fermentation

The bacterial counts observed in the monoculture, generally was lower than the counts in mixed culture fermentation. Bacterial counts in watermelon was between 3.0-4.3±0.3 log (cfu) mL^{-1}, while yeast counts was between 4.1±0.2-4.9±0.3 log (cfu) mL^{-1}

The watermelon-banana mixture medium had bacterial counts of between 2.6-4.3±0.3 log (cfu) mL^{-1} and yeast counts of between 4.6-4.9±0.1 log (cfu) mL^{-1} sequentially for the period of fermentation. The watermelon-pineapple mixture medium had the lowest bacterial and yeast counts of between 2.5±0.1-4.3±0.1 and 4.6-4.7±0.1 log (cfu) mL^{-1}, respectively (Table 3, 4).

Alcohol content (%) yield was however absent from the watermelon mixed culture fermentation until 48 h of fermentation where 0.4% of alcohol was recorded. There after, it increased slowly to 1.12% at termination of experiment. The watermelon-banana mixture yielded alcohol content of 2.4% after 24 h of fermentation and 4.08% at termination of experiment. Also, the watermelon-pineapple mixed culture fermentation yielded alcohol content of 2.8% after 24 h of fermentation which increased gradually to 5% at termination of experiment (Table 5). In the monoculture fermented samples, the watermelon medium yielded alcohol content of 1.2% after 24 h of fermentation and increased to 2.6% at termination of experiment. Watermelon-banana mixture also yielded alcohol content of 0.65% which increased to 5.11% at end of experiment while watermelon-pineapple mixture yielded 0.10% which as well increased to 6.2% at the end of experiment (Table 6).

Table 7: Means sensory score of the alcoholic beverage sample

Treatments sample code	Colour	Taste	Aroma	Overall acceptability
Wmo	5.8c	5.7c	5.7c	5.6c
Wmi	6.2b	5.3c	5.2c	5.3c
WBmo	7.4b	7.6b	7.4b	7.5b
WBmi	7.3b	7.0b	6.3b	7.2b
WPmo	8.4a	8.8a	8.8a	8.6a
WPmi	8.4a	8.6a	8.3a	8.2a

Wmo = Watermelon monoculture fermented, Wmi = Watermelon mixed culture fermented,
WBmo = Watermelon Banana monoculture fermented, WBmi = Watermelon Banana mixed culture fermented,
WPmo = Watermelon Pineapple monoculture fermented, WPmi = Watermelon Pineapple mixed culture fermented
Values with different letters are significantly different at $p<0.05$

Sensory Evaluation of the Samples

The mean sensory analysis of the fermented alcoholic beverages, showed significant difference ($p<0.05$) in colour, taste, aroma and overall acceptability. The alcoholic beverages produced from the combination of watermelon-pineapple mixture were the most favoured, having between 8.2-8.8 ranking. The watermelon-banana mixture was followed with rankings of between 6.3-7.6 while the beverages from watermelon alone were of unacceptable characters in all the parameters evaluated, with low rankings from 5.3-6.2 (Table 7).

DISCUSSION

Yeasts and bacteria species were isolated during the production of alcoholic beverage using the tropical fruits. Molds were not isolated due to low level of oxygen as the fermentors were air tight. Similar observation was also reported by Odunfa (1981). It could as well be due to the increasing acid content produced by lactic acid bacteria. The fruits juice provided all the nutrients and natural conditions necessary in the completion of the fermentation by microorganisms, hence the fermenting musts were not induced with any artificial nutrient. The result of this work correlate with Ogbona (1993) who fermented banana and palm-wine with indigenous microflora and obtained quality wine product. Hence, microorganisms are able to make use of carbohydrates such as fructose, galactose, lactose, maltose and mannitol as a source of energy, it is therefore expected that some components of the fruits, most especially sugars might have affected the relative growth rate of the different species of the isolated yeasts and bacteria. It has been observed that yeasts make use of sugars as their main source of energy and this could account for the ease at which the various sugars present in the tropical fruits were utilized. This could result to the lower bacterial and yeasts counts encountered in watermelon + banana and watermelon + pineapple beverages, than the beverage produced from watermelon, as it was of lower sugar content. Despite the variation in microbial counts in the various fermented substrates, a general high microbial count was recorded. This observation of the total plate counts result, may be due to the high water activities (a_w) which enhanced the microbial prolific successions in the various fermented substrates.

All the stages involved in the alcoholic beverage production, contributed successfully to the attainment of a quality and desired alcoholic beverage from the tropical fruits. The crushing of the raw materials for the juice extractions served an access to high microbial involvement in both the mixed and the monoculture fermentations.

Some of the beverage qualities in this crude forms, were desirable as determined by variance analysis. If enhanced with some supplements, the product quality could be improved. And due to the high microbial counts during fermentation, the alcoholic beverages could be liable to spoilage in a short time, if adequate storage facilities are not provided. And, also, due to the low alcoholic contents of the beverages, chemical or physical preservation could be required to ensure storage safety.

The various alcoholic beverages produced showed characteristic differences among the treatments. This observation is as a result of the fruits components and the fermentation methods used, thus,

seeding with strains of yeast and the spontaneous fermentation method used did characterized differences among the different treatments. This is evidence, as watermelon produced alcoholic beverage of which natural aroma was not prominent could not be rated of better characteristics and acceptable than the mixed fruits produced beverages of highly natural flavour and taste, distinguishing the chemical components and organoleptic differences observed. It cannot be ignored that the species of organisms involved in the spontaneous fermentation are influenced by the source of raw materials used for the beverage production and the composition of the fermenting mixtures. Utilizing these fruits for beverages by individuals, would not lack the quality control which could fail to meet microbiological safety and other quality standards if only care could be managed over human contaminations.

The qualities of the beverages as notice was on the trend of the components of the natural colour, taste and flavour of the fruits used. This was however, the unique blend and overall acceptance the banana mixed and pineapple mixed had over the watermelon produced beverage samples.

The monoculture fermented alcoholic beverages were found better than the mixed fermented alcoholic beverages in taste aroma, odour, colour and overall acceptability. This findings support the previous findings of Smhitte *et al*. (1984) who emphasized on the preference of wine produced with selected yeast. Also, mixture of fruits for the production of alcoholic beverages as noted in this finding, is more accepted because of its better quality compared to the single fruit fermented beverage. However, fruits of higher sugar contents for a better blend and quality beverage.

The low alcohol yield at the initial state of fermentation could be due to the types of microorganism present and their ability to break down some of elements on the must. Meanwhile, it must be understood that for juice to become wine or alcoholic beverage, fermentation must occur by degrading the fermentable sugars to lactic acid by yeast species and lactic acid bacteria. This condition however, resulted to the gradual increase in alcohol yield as observed in the fermented samples.

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