STUDIES ON WINE PRODUCTION FROM ORANGE (*CITRUS SINENSIS*)

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ABSTRACT

Wine was produced from orange must (*Citrus sinensis*) at 1:4 orange must: water (A) and augmenting with sugar solution at 1:4 orange must: sugar solution (B) using the normal flora. On fermentation for 72h, wine from A had average pH of 3.6, optical density of 0.5148, specific gravity 0.994, % alcohol of 1.355, temperature of 29.4^{0}C, % titratable acidity of 0.3, total aerobic count of 10.4 $\log_{10}$cfu/ml, fungal count of 10.16 $\log_{10}$cfu/ml and R_f of 4.05cm. Wine from B had average pH of 3.5, optical density of 0.6448, specific gravity of 1.009, % alcohol of 1.356%, temperature of 29^{0}c, % titratable acidity of 0.89%, total aerobic count of 9.054 $\log_{10}$cfu/ml, fungal count of 10.58 and R_f of 4.2cm. Taste assessment showed smooth sweetness with characteristic orange flavor after 48h and a reduction of the yellow coloration and sweetness after 72h of fermentation. There were no statistically significant differences at 95% confidence level between the two batches of orange wine. Thus, wine could be produced from orange juice using the natural flora or by augmenting with sugar, if higher residual yeast load is desired, for immediate consumption after 48h of fermentation, without chemical preservatives and/or pasteurization.

Key words: Orange, Flora, Fermentation, Sugar, Wine, Flavor, Yeast.

INTRODUCTION

Orange is the fruit produced by orange tree (*Citrus sinensis*). It is a small flowering tree growing to about 10m tall with evergreen leaves, which are arranged alternatively, of ovate shape with crenulate margins and 4-10cm long. The orange fruit is hesperidium (Amerine *et al.*, 2007).

Orange juice is one of the commodities traded on the New York Board of Trade. Brazil is the largest producer of orange juice in the world, followed by USA. It is made by squeezing the fruit on a special instrument called a "Juicer". (Amerine and Kunkee, 2005).

Wine is an alcoholic beverage typically made from fermented grape juice. The natural chemical balance of grape is so complete that they can ferment naturally without the addition of sugar, acids, enzymes or other nutrients. However, any fruit with a good proportion of sugar may be used for wine production, and the resulting wines are normally named after the fruit hence banana, apple, orange, pineapple, strawberries and coconut may be used to produce wine. These types of wines are also referred to as fruit wine (Alexander and Charpeater, 2004). Wine is produced by fermenting crushed fruits of choice using various types of yeast. Yeast consumes the sugar present in the fruit juice producing alcohol and carbondioxide as by-products. The type of wine to be produced dictates the fruit and the strain of yeast to be involved (Alexander and Charpenter 2004). Fermentation is the partial breakdown of organic compounds generating energy in the form of Adenosine triphosphate (ATP) by substrate level phosphorylation using organic compound as both election doctor donor and acceptor (Uraih, 2003).

Wine plays almost indispensable roles in the life of man ranging from social function, religious rites/rituals as well as economic benefits to producers and merchants. After woman, man's oldest love has undoubtedly been wine. Indeed, some event put it first and quote man's three joys as being "wine, woman and song" (Alexander and Charpeater 2004).

The attendant health fear in the use of chemical preservatives has necessitated the search for safer means of production of foods without chemical additives. This study is aimed at producing wine from orange for immediate consumption and possibly preservation by refrigeration whenever the need arises.

MATERIALS AND METHODS

Collection of materials: Ripe orange fruits (*Citrus sinensis*) were purchased from Abraka market in Delta State, Nigeria and washed with tap water in the laboratory.

Preparation of sugar solution: Clean water was boiled for five minutes and allowed to cool. One (1) teacup-full of granulated sugar was dissolved in one litre of water to obtain the sugar solution.

Preparation of must juice: This was carried in accordance with the method of Ibeh and Uraih (2000)

Fermentation of orange juice (must): This was carried out using a modification of the method of Uraih (2003). Two (2) sterilized 10L- plastic buckets were aseptically filled with 1.5 L of must. To the first bucket (A) 6 L of cooled distilled water was added while 6 L of cooled sugar solution was added to B. The buckets were properly covered and allowed to ferment for 72h at room temperature.

Ripe unbruised orange fruit

↓

Washed with sterile water and detergent then rinsed

↓

Fruits were peeled

↓

Cut into pieces and blended into slurry

↓

7.5 L of blended slurry (A)

or

Addition of 6.0litre of sugar solution to 1.5litre of blended slurry (B)

↓

Fermentation vessel was covered

↓

Fermentation at 30 ± 2^0c for 72h

↓

Orange wine produced

Fig 1: *Flow Chart of orange wine production.*

Determination of temperature: This was carried out using a 0-100^{o}C thermometer after one hour and 24-hourly for 72h.

Determination of pH, Optical density $_{560nm}$, % titratable acidity and % alcohol: These were carried out in accordance with the methods of Kunkee and Amerine (2002) after one hour and 24-hourly for 72h.

Determination of specific gravity (SG): This was carried out in accordance with the method of Fawole and Oso (2008).

Determination of Retention front (R_f): This was carried out in accordance with the method of Ogunkoye and Olubayo(1977).

Determination of total aerobic and fungal counts: These were carried out in accordance with the methods of Cowan and Steel (2004) after one hour and thereafter 24-hourly for 72h.

Fungal Isolation and identification: These were carried out in accordance with the methods of Harrigan and McCane (2001) at 48h of fermentation.

RESULTS AND DISCUSSION

The changes in temperature during orange wine production presented in Fig 2 shows that there was a decrease from 1h to 24h corresponding to an increase in OD (Fig 3), TAC and TFC (Fig 6) and a decrease in SG (Fig 5). This was followed by a sharp increase in 48h followed by a slight increase in 72h. There were corresponding changes in OD, SG, TAC and TFC. The average temperatures of 29.4^{o}C and 29^{o}C for A and B respectively (Table 1) as well as no statistically significant differences (Table 3) account for no detectable differences in taste and organoleptic properties (Table 2). These results agree with reports of previous workers (Kunkee and Amerine, 2000; Okafor, 2007).

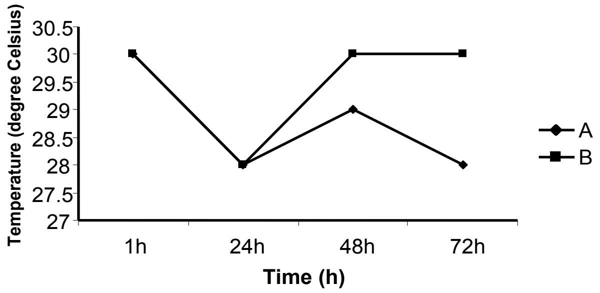


Fig 2: *Changes in temperature of orange wine*

Key: A = Natural fermentation. B = A + Sugar.

The changes in optical density during orange wine production presented in Fig 3 shows that there was an increase from 1h to 48h and a decrease in 72h corresponding to changes in temperature (Fig 2), SG (Fig 5) and TAC and TFC (Fig 6). Fruit juice provide good medium for fungal growth – low pH, high redox potential, high water activity (Aw) and low content of vitamin B, all of which represent a hurdle for bacterial growth except acid-tolerant species like lactic acid bacteria (LAB). The average values of 0.5148 and 0.6448 respectively for A and B were not statistically significant (Table 1). These results agree with previous reports (Porkon, 1995; Okafor, 2007).

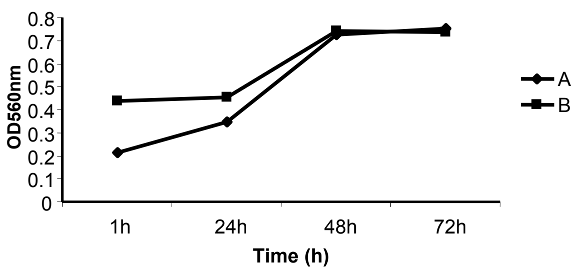


Fig 3: *Changes in Optical Density of orange wine*

Key: A = Natural fermentation. B = A + Sugar.

The changes in pH and % TA during orange wine production presented in Fig 4 show that while there was a decrease in pH from 1h to 48h and an increase thereafter to 72h for A, there was increase in pH from 1h to72h for B corresponding to changes in % TA (Fig 4). The further decrease in pH and increase in % TA after 48h as well as the R_f values of 4.05 and 4.2 for A and b respectively (Table 1) could be due to malo-lactic fermentation. The average values of pH and % TA were not statistically significant (Table 1). These results agree with reports of previous workers

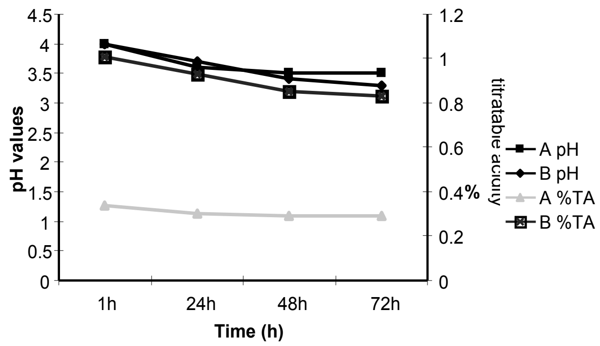


Fig 4: *Changes in pH and % titratable acidity of orange wine*

Key: A = Natural fermentation. B = A + Sugar.

The changes in SG and % alcohol during orange wine production presented in Fig 5 shows no differences in A and B as the SG decreased from 1h to 72h while the % alcohol increased from 1h to 48h and decreased thereafter in 72h. There were corresponding changes in TAC and TFC (Fig 6) indicating microbial succession due to malo-lactic fermentation. The average values of SG and % alcohol (Table 1) were not statistically significant. These results agree with previous reports (Amerine *et al.,* 2007; Okafor, 2007; Madigan *et al.,* 2009).

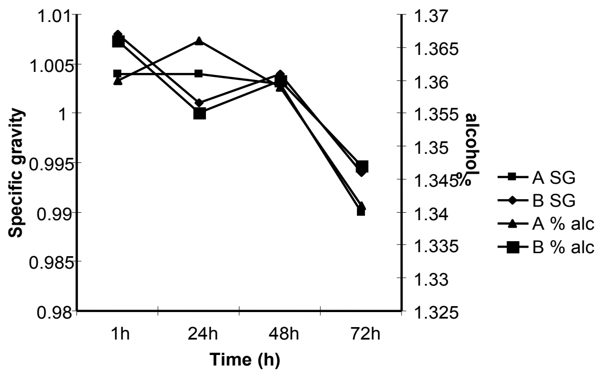


Fig 5: Changes in SG and % alcohol of orange wine

Key: A = Natural fermentation. B = A + Sugar. OD = Optical density.

The changes in TAC and TFC during orange wine production presented in Fig 6 shows that both followed the same pattern of increase from 1h to 48h but while there was a slight increase in 72h for A, B had a slight decrease. This could be due to microbial succession and/ or exhaustion of nutrients. However, A had a higher residual microbial load at 72h than B. average values of TAC and TFC (Table 1) were not statistically significant. These results agree with previous reports (Porkon, 1995;

Amerine and Kunkee, 2005; Robinson, 2006; Amerine *et al.,* 2007; Anon., 2010).

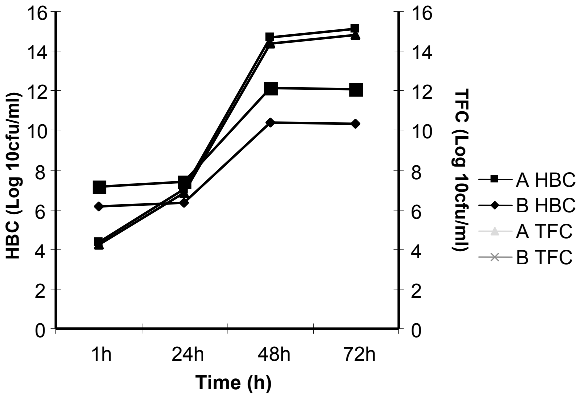
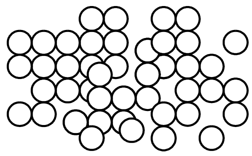


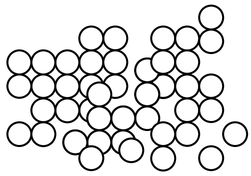
Fig 6: *Changes in HBC and TFC of orange wine*

Key: A = Natural fermentation. B = A + Sugar. %TA = % Titratable acidity.
TAC = Total aerobic counts. TFC = Total fungal counts

The average values of tested parameters during orange wine production presented in Table 1 show no statistically significant differences in the tested parameters. This is further corroborated by the absence of detectable differences in microscopy (Fig 7), absence of organoleptically detectable and taste differences between A and B (Table 2) and no statistically significant differences at 95% confidence level (Table 3).



a. Yeast isolated from A



b. Yeast isolated from B

Fig 7: *Microscopy of Yeast Isolates*

Table 1: Average values of tested parameters

Parameters	A	B
pH	3.6	3.54
Temperature (^{0}C)	29.4	29
Specific gravity	0.994	1.009
Optical density	0.5148	0.6448
% titratable acidity	0.3	0.89
% Alcohol	1.355	1.356
Total aerobic count (log_{10}cfu/ml)	10.4	9.05
Fungal count (log_{10}cfu/ml)	10.16	10.58
R_f at 72h	4.05	4.2

Key: A = Natural fermentation. B = A + Sugar. R_f = retention front.
t-stat = 0.01287, t-crit = 1.7459 at 95% confidence level.

Table 2: Changes in taste and color of orange wine during fermentation

Fermentation time	A	B
24h	Yellow color, foaming/ bubbling, sweet in taste and possessed pronounced orange aroma	Yellow color, foaming/ bubbling, sweet in taste and possessed pronounced orange aroma
48h	Yellow color, higher foaming/ bubbling, sweet in taste and possessed pronounced orange aroma	Yellow color, higher foaming/ bubbling, sweet in taste and possessed pronounced orange aroma
72h	Reduction in yellow coloration and sweetness but retained the characteristic orange aroma	Reduction in yellow coloration and sweetness but retained the characteristic orange aroma

Table 3: Statistical analysis (t-test)

Parameter	t-stat	t-crit
pH	0.2526	1.9432
Temperature	-1.4316	2.1319
OD	-0.2163	2.1319
SG	-0.1236	2.1319
% alcohol	0.1596	2.1319
% Titratable acidity	-14.013	1.9432
Total aerobic counts	1.0995	2.1319
Total fungal counts	0.1189	1.9432

CONCLUSION

It is feasible to produce wine from orange juice for consumption after 48h of fermentation. No chemical preservatives are needed as the wine could be stored by refrigeration. For higher desirable residual yeast load, the addition of granulated sugar to the juice at 1:4

prior to fermentation with the innate wild yeast could be employed. The orange wine would however loose its characteristic orange yellow coloration and sweetness after 48h of fermentation. There were no statistically significant differences at 95% confidence level in the tested physical and chemical parameters as well as taste and color between the two batches of orange wine produced. Wine can thus be produced from orange juice at home by individuals without the need for chemical preservatives and/or pasteurization.

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