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Full Length Research Paper

Quantitation of alcohols in orange wine fermented by four strains of yeast

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Gas liquid chromatography was used to identify the types of alcohol present in wines produced by fermenting orange juice with four strains of yeast; *Saccharomyces cerevisiae* (isolated from yam), *S. cerevisiae* (from sugarcane molasses), *Saccharomyces carlsbergensis* (from sugarcane molasses) and *S. cerevisiae var. ellipsoideus* (from orange juice). Ethanol and methanol were the predominant alcohols. The ethanol content was highest, 90.38% with *S. cerevisiae var. ellipsoideus* and least, 81.49% with *S. cerevisiae* (from sugarcane molasses). The methanol concentration varied between 9.51% with *S. cerevisiae var. ellipsoideus* and 14.93% with *S. carlsbergensis*. The other alcohol detected, isopro-panol was generally negligible; 0.10 to 0.25% except with *S. cerevisiae* (from sugarcane molasses) which produced it as 5.46% of the total alcohol. The total alcohol was highest, 6.50 ± 0.15% with *S. carlsbergensis* and least 3.23 ± 0.12 with *S. cerevesiae var. ellipsoideus*.

Key words: Alcohol types, fermentation, orange wine, *Saccharomyces*, yeast strains.

INTRODUCTION

Tropical fruits have been used as substrates for the production of wines (Maldonado et al., 1975; Anuna et al., 1990; Ndip et al., 2001; Osho, 2005; Okunowo et al., 2005). However, the alcohol profiles, types and quantities were not stated in the majority of these reports. Various factors influence the fermentation process and determine the end products obtained. These include substrate rela-ted factors such as cultivar types, cultivation conditions, conditions at harvest and post harvest handling (Daudt and Ough, 1973; Bell et al., 1979; Liu, 2002; Jonathan and Errol, 2000; Joshi and Sandhu, 2000; Kourkoutas et al., 2005). Though the fermentation of fruit sugar usually yields ethanol as the predominant alcohol, small quan-tities of other higher alcohols (referred to as fusel oil) are also produced from the oxidative deamination, decar-boxylation and reduction of amino acids and sugar degra-dation (Anuna and Akpapunam, 1995) . The presence of pectin in some fruits may also result in methanol genera-tion in the fermenting wort (Anuna and Akpapunam, 1995). The alcohol profile is a significant factor in the quality of wines. (Drawert and Rapp, 1966; Anuna and Akpapunam, 1995).

Yeast species are used in many industrial fermentation processes including alcoholic beverages production. The quality of wine produced greatly depends on the yeast strain (Kunkee, 1984; Okunowo et al., 2005). Development of improved starter organisms for fermentation of citrus juice may offer a relative simple avenue for reducing post harvest wastage of citrus fruits in low utilization environment and in places where the production of citrus concentrates is low or non existent. Juice concentrates are readily storable and can be used for production processes even when the fruit is out of season (Ramachandra and Arun, 2005; Siddik et al., 2006). For example, in India alone an estimated loss of around 35 000 million Indian Rupees (around 638 million US $) worth fruits and vegetables was recorded despite the fact that India produces around 60 to 65 million tons of fruits and vegetables (Ramachandra and Arun, 2005) . While reports indicate that about fifty percent of citrus fruit goes to waste in Nigeria (Jennifer, 1999). In an effort aimed at increasing the low industrial utilization and reducing the high wastage of orange (*Citrus sinensis*) fruits in the developing world, we investigated the possibility of exploiting the fermentative ability of yeasts to produce orange wines (Okunowo et al., 2005).

In this present study, the types and quantities of alcohol present in orange wines produced from musts fermented by four strains of *Saccharomyces* species were determined.

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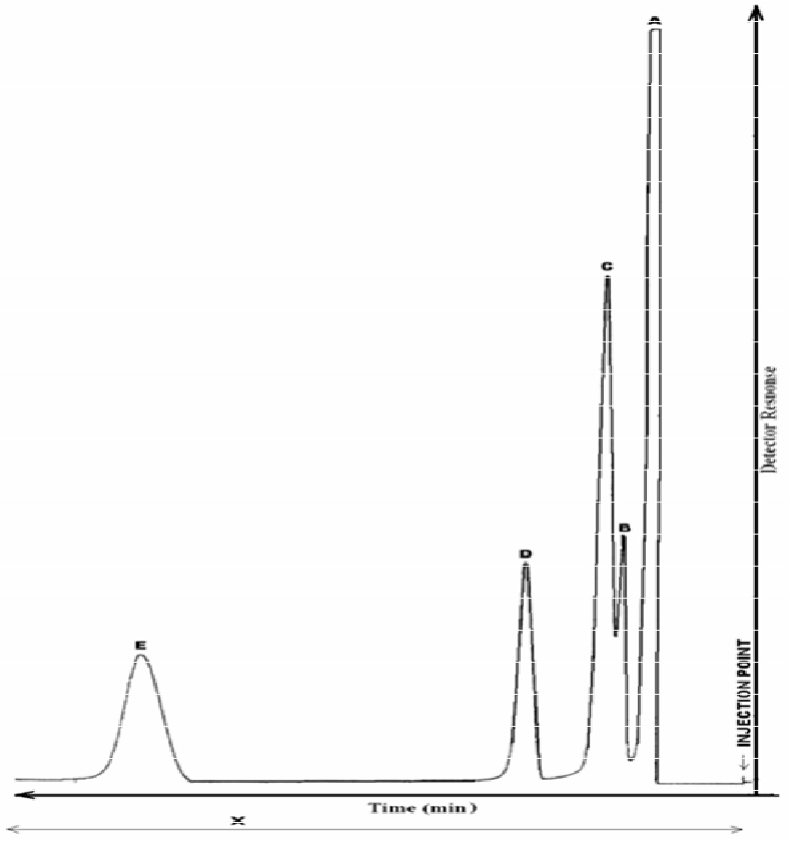

Figure 1. Alcohol profile of standard alcohol mixture. Attenuation condition: 64 x 10^2 (100mV x 2). A = Acetone + Isopropanol, B = Methanol, C = Ethanol, D = Isoamyl alcohol and E = Butanol.

MATERIALS AND METHODS

Microorganisms

The yeast strains used in this study were obtained from the stock cultures maintained at the Federal Institute of Industrial Research Oshodi (F.I.I.R.O), Lagos, Nigeria. They were identified as *Saccharomyces cerevisiae* (isolated from sugarcane molasses), *S. cerevisiae* (isolated from yam), *S. carlsbergensis* (isolated from sugarcane molasses) and *Saccharomyces cerevisiae var. ellipsoideus* (isolated from orange juice). The organisms were subcultured aerobically for reactivation and increased biomass concentration at pH 4.5, 30°C for 24 h in a medium containing (g/l): yeast extract, 3; peptone, 5; malt extract, 5 (Nigam et al., 1998). The cells were harvested by centrifugation at 1600 g for 5 min and washed with 0.85% NaCl solution. These steps were carried out under sterile conditions. The cells obtained were used as the starter cultures.

Preparation of the fermentation medium

The orange fruits *(C. sinensis*) were purchased at a local market in Lagos. They were thoroughly washed with 0.1% of sodium metabisulphite solution, cut into pieces and pressed manually to obtain the juice. The juice was sterilized with 200 mg/L sodium metabisulphite and allowed to clarify at –5°C for 24 h. The supernatant was analyzed for the total soluble solid and the pH. The total soluble solid was fortified with sucrose from 12.5 to 18 ° Brix to give enough fermentable sugar.

Fermentation of the wort

Fermentation experiments were performed according to Okunowo et al. (2005) in 1L glass batch reactor system (Biostat M, B. Braun Biotech International, Germany) equipped with an agitator. After cleaning by steam sterilization at 121°C for 15 min, the fermenter was filled with 750 ml of the fermentation medium with the addition of the following nutrients (g/l): diammonium hydrogen phosphate, 0.5 g; magnesium sulphate ($MgSO_4.7H_2O$), 0.2 g and urea, 0.5 g (Nigam et al., 1998). One drop of antifoam A (Sigma Chemical Co., London) was added to each reactor unit to prevent foaming. Each reactor was inoculated with 1% (w/v) of the yeast strains. The fermentation was allowed to proceed at room temperature (25 ± 2°C) for five days. The agitation speed was maintained through out the experiment at 100 rpm for even distribution of the yeast and the nutrient respectively. The wine was clarified with 0.1% bentonite, racked and stored at 2°C until analyzed.

Quantification of total alcohol

The total alcohol of the wine samples was determined by the specific gravity method (A.O.A.C, 2000). The percentage (w/v) total alcohol was presented as mean ± SEM of triplicate results from three fermentation tanks.

Gas-liquid chromatography of alcohols

At the end of the fermentation process, one hundred milliliters of the

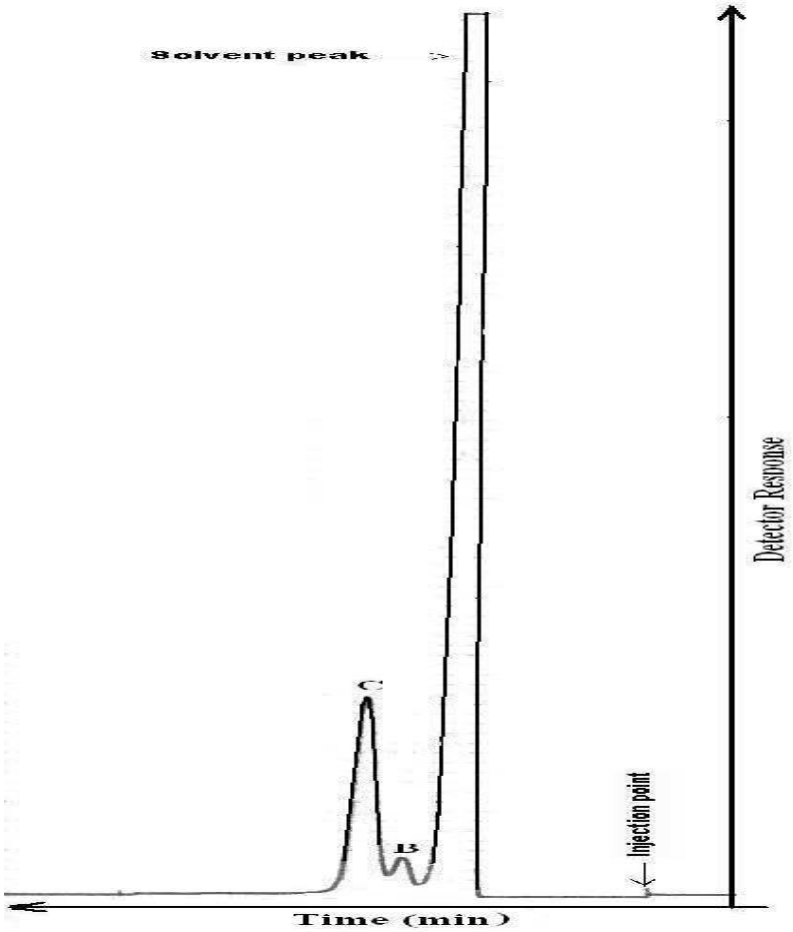

Figure 2. Alcohol profile of wine sample mixture from four yeast strains. Attenuation condition: 64 x 102 (100mV x 2). B = Methanol and C = Ethanol.

wine was steam distilled and distillates analyzed for alcohols. A flame ionization detector (H_2, 40 ml/min; air, 600 ml/min.) was used with a gas-liquid chromatograph series 204 (Pye Unicam, Cambridge Scientific Instrument Co., UK). A glass column (2.1 by 2.75 mm internal diameter) was packed with 10% carbowax 20M on Chromosorb WHP of mesh size 100/120 (Pye Unicam, Cambridge Scientific Instrument Co., UK). The carrier gas was nitrogen at the flow rate of 25 Kg/cm^2. The fractionation was carried out in an isothermal temperature. The oven temperature was 78^{O}C, the sample size was 1μL and the detector temperature was 120^{O} C. The attenuation condition was varied between 64 x 10^2 (100mv x 2) and 64 x 10^2 (10mv x 1). Peaks were identified by comparison of retention times with those of standard alcohols, methanol, ethanol, amyl alcohol, isopropanol and 2-butanol.

Statistical analysis

The data presented in this study are results obtained from three fermentation flasks. This was expressed as mean ± S.E.M of triplicate results.

RESULTS

The standard alcohols were mixed using acetone and the chromatogram obtained is as shown in Figure 1. Five types of alcohol standards were mixed but only four distinct alcohol peaks (B, methanol; C, ethanol; D, isoamyl alcohol and E, 2-butanol) were observed in the chromatogram. It thus appeared that peak A was an overlap between Isopropanol and the solvent; acetone. The four orange wines produced using the different yeast strains were mixed and subjected to the same attenuation condition (100mV x 2) as that of the standard mixture. This was done to give an idea of the alcohol types in the wines. Two clear and defined peaks corresponding to ethanol and methanol were seen (Figure 2). When the atenuation condition was varied on the alcohol standard mixture, 64 x 10^2 (10mV x 1) yielded the best result. Under this condition, the acetone and the isopropanol had a different retention time in the standard alcohol mixture and five different peaks were observed (Figure 3). The same retention time was also observed when individual standard alcohol was injected to the gas chromatograph at this same attenuation condition. When the fractionation of the wine samples obtained from the yeast strains was carried out at 64 x 10^2 (10mV x 1) three different clear peaks with corresponding retention time to that of isopropanol,

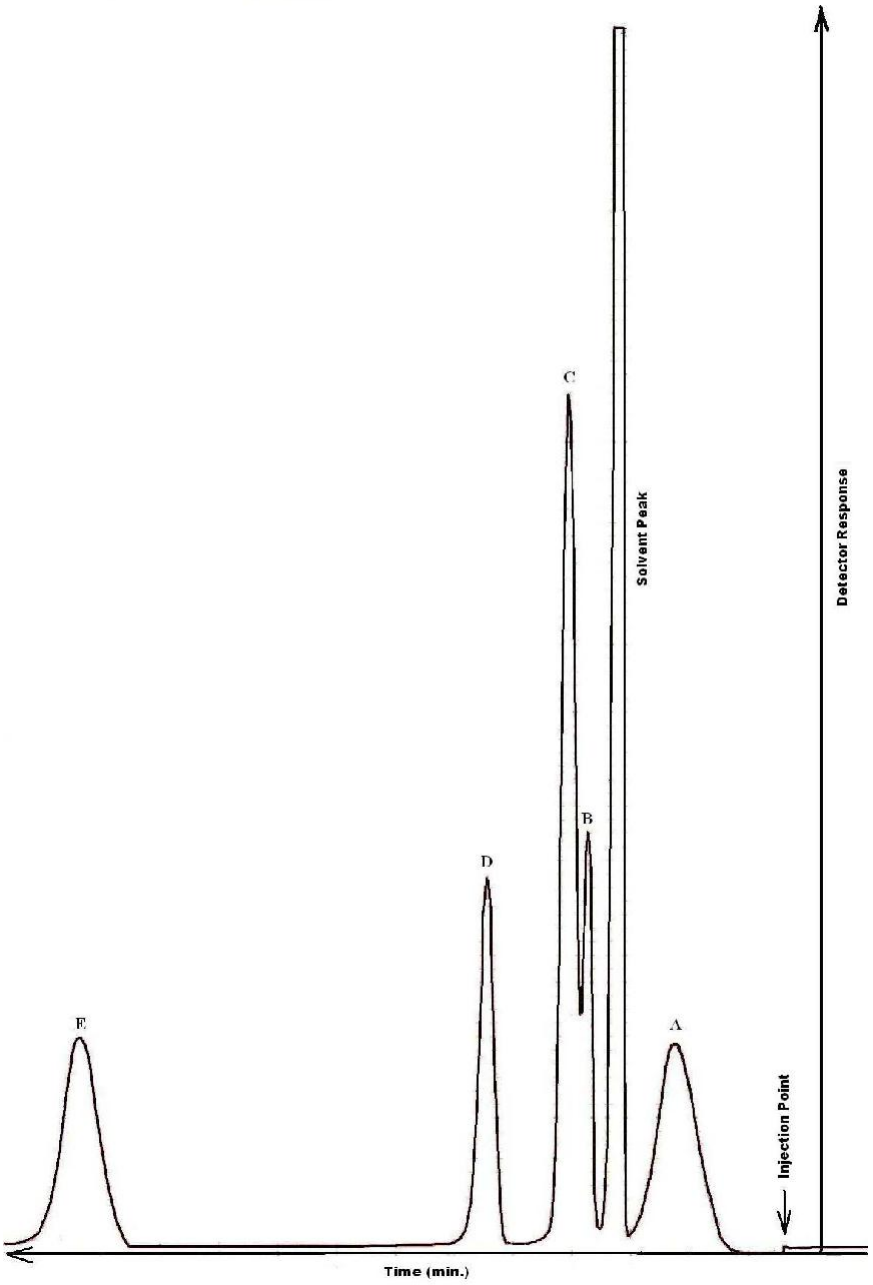

Figure 3. Alcohol profile of standard alcohol mixture. Attenuation condition: 64 x 102 (10 mV x 1). A = Isopropanol, B = Methanol, C = Ethanol, D = Isoamyl alcohol and E = Butanol

methanol and ethanol on the standard alcohol chromatograms were obtained and the result (area %) is as presented in Figure 4. The proportion of each alcohol type as a percentage of the total alcohol in the wine sample was calculated. The results show that the quantity of ethanol was the highest and that of isopropanol was negligible in the wine samples (Figure 4). The yeast strains produced varying amount of total alcohol in the wine samples (Figure 5). This was highest, 6.50 ± 0.15% with *S. carlsbergensis*; 5.73 ± 0.19% with *S. cerevisiae* (yam); 4.72 ± 0.09 with *S. cerevisiae* (sugarcane) and least, 3.23 ± 0.12 with *S. cerevesiae var. ellipsoideus*.

DISCUSSION

An appreciable amount of total alcohol in the wine samples were produced by the yeast strains in a trend that is similar to that obtained in our previous work (Okunowo et

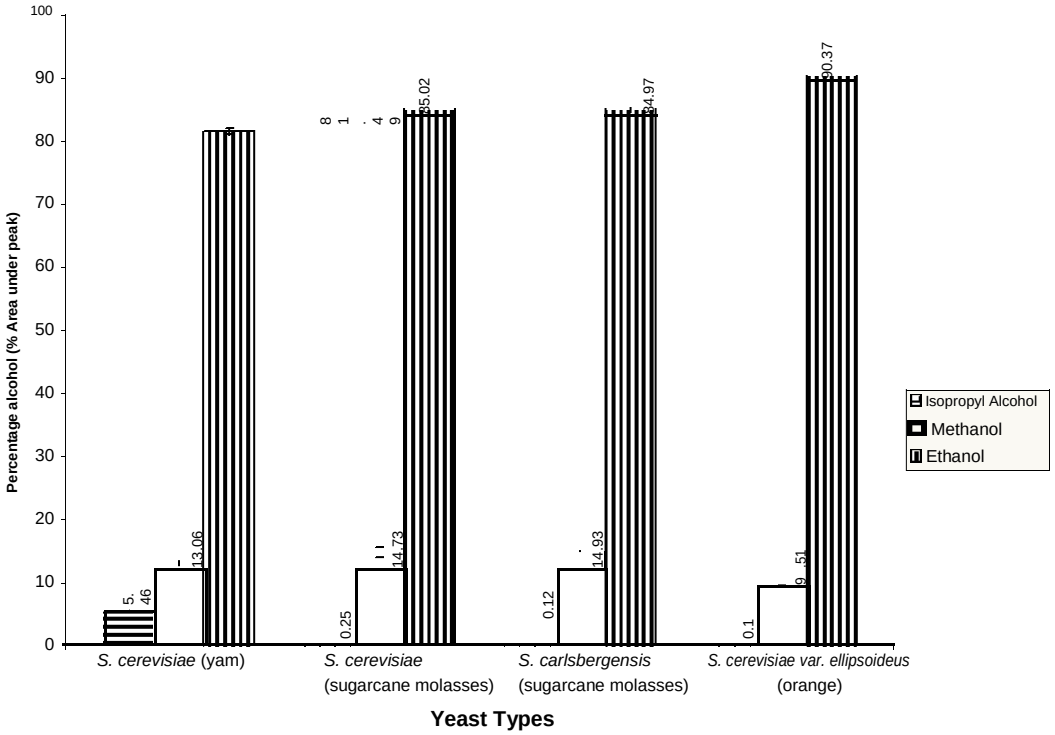

Figure 4. Alcohol profile (As % of total alcohol) of wine samples fermented by four strains of yeast. Values are mean ± SEM for triplicate results.

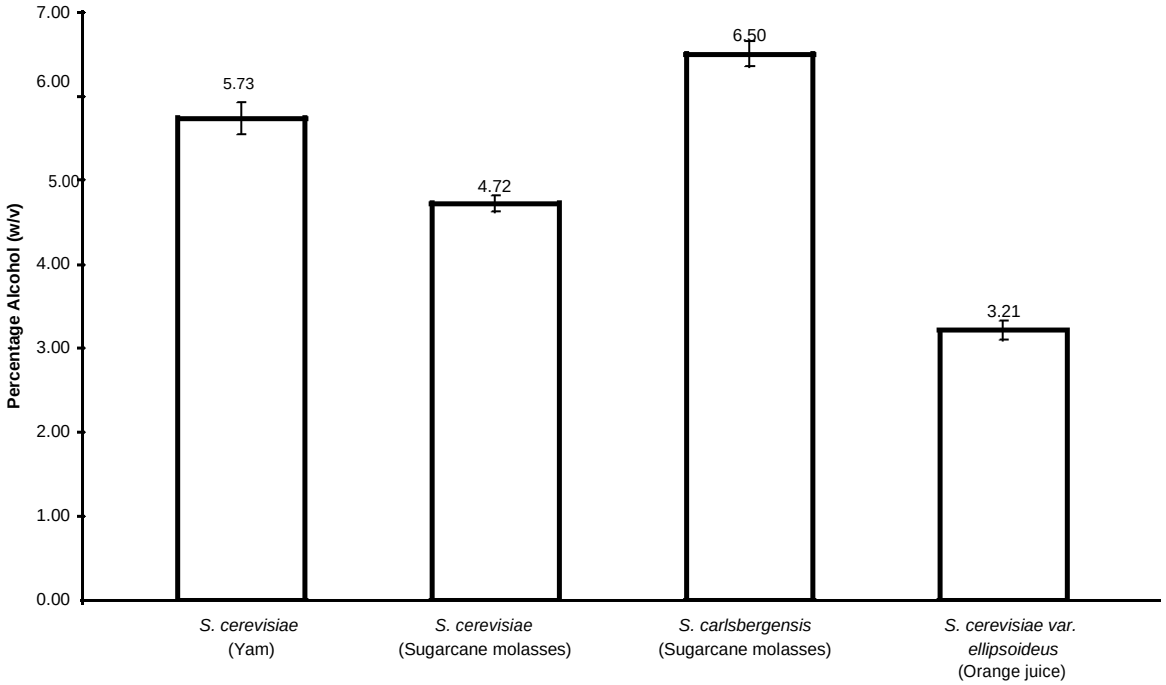

Figure 5. Percentage total alcohol in wines produced by four yeast strains. Values are mean ± SEM for triplicate results.

et al., 2005). The methanol content of the wines was also appreciable. Methanol, a toxic alcohol, is formed during fermentation by the hydrolysis of naturally occurring pectin in wort (Tomoyuki et al., 2000). The methanol

content was least, 9.51% (area %), and highest, 14.93% (area %), in the wine sample produced by *S. cerevisiae var. ellipsoideus* and *S. carlsbergensis* respectively. The production of methanol may be reduced by degrading the pectins with the addition of pectolytic enzymes prior to the fermentation process. These enzymes degrade pectin and reduce the viscosity of the solution or medium to be fermented so that it can be handled easily by the fermenting organism since more fermentable sugars are released (Naidu and Panda, 2004). Although undesirable in wines, methanol has a wide application in the industrial and the health sector. Thus, the high methanol producing yeast may have other potential uses rather than in wine production.

The quantity of the most predominant alcohol, ethanol was between 81.49% (area %) and 90.38% (area %) for wine produced by *S. cerevisiae* (from sugarcane molasses) and *S. cerevisiae var. ellipsoideus* respectively. Although, *S. cerevisiae var. ellipsoideus* produced the least amount of total alcohol in our previous study (Okunowo et al., 2005) . The result of our present study implies that this organism is the most efficient in ethanol production.

The amount of isopropanol was generally negligible in the samples except in the wine produced by *S. cerevisiae* (sugarcane molasses) where it was 5.46% (area %) as shown in Figure 4. In general, the wines contained low amount of fusel oil (C_3 – C_4 alcohol). This may be due to a paucity of amino acids in the orange juice. Yeasts degrade most amino acids by a process which involves deamination and decarboxylation followed by reduction to yield an alcohol (fusel oil) containing one carbon atom less than the original amino acid (Dickinson et al., 2003; Schoodermark-Stolk et al., 2005).

Fermentation of the orange juice by *S. cerevisiae* from yam and *S. cerevisiae* from sugarcane molasses resulted in products with different concentrations of alcohol types despite the fact that the fermenting organisms are of the same species (Figure 4). This indicates that the source of the yeast may influence the alcohol profile of the wine produced. It is therefore concluded that the source of the yeast is thus an important factor in the determination of the amount and types of fusel oils present in wines and hence the quality of the wine.

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