Original Research Article

Evaluation of the Prevalence of Congeners from Distilled Spirits of Different Sources

ABSTRACT

Aim: With recent increase in health-related incidence arising from consumption of spirit beverages in Nigeria, there is need to investigate the prevalence of possible contaminants in spirits that may have toxicological effect on human when consumed. The purpose of this study was to determine the type and levels of congeners present in spirits obtained from fermentates of cassava, molasses and palm wine purchased from different locations.

Study design: This study was made to fit a one way Analysis of Variance.

Place and Duration of Study: The research was carried out at laboratory of Department of Food Science and Technology, Federal University of Technology, Owerri and Project Development Agency (PRODA) Enugu and International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria, between April 2017 and November 2018.

Methodology: Analysis of common congeners in spirits of cassava, molasses and palm wine fermentates obtained through distillation method was carried out using the Gas-Chromatography. Physicochemical properties of the spirits measured were specific gravity, pH and viscosity. The spirit distillates were analysed for concentrations of ethanol, higher alcohols and possible congeners such as esters, ethyl carbamate (EC) and ethyl acetate.

Results: The distillates yielded alcoholic content of 39.00 to 46.71%. Ethyl carbamate content of spirits from cassava recorded an average mean value of 13.44µg/l which was not significantly different from ($P > .05$) spirits from molasses and palm wine, with an average mean values of 12.49µg/l and 13.75µg/l respectively. The most important higher alcohols of the spirit distillates responsible for aromatic characteristic of spirits were found to be 1-propanol (0.06-0.11%), isobutyl alcohol (0.02-0.09%) and isoamyl alcohol (0.12-0.76%). The type and location of raw materials did not significantly affect the concentrations of the available congeners found in the distilled spirits. Good fermentation employed in this research work significantly reduced the concentrations of the detected congeners.

Conclusion: Comparing the results with data from literature, it can be concluded that the concentrations of all investigated volatile compounds in the samples of spirits from cassava, palm wine and molasses are commonly acceptable. Federal regulatory agencies such as National Agency for Food Drug Administration and Control (NAFDAC), Standard Organization of Nigeria (SON) should be encouraged to carry out routine analysis on commonly produced and sold spirit beverages in order to prevent sale of contaminated drinks.

Keywords: congeners, distillates, ethyl carbamate, fermentates, spirit beverages

1. INTRODUCTION

Spirits are alcoholic beverages with high ethanol contents made by distillation of fermented mashes derived from fruits, cereals, root crops, sugar cane or other sources of fermentable sugars [1]. Alcoholic beverages that have lower alcohol content are produced by fermentation of sugar or starch-containing plant materials. Plant materials such as sugar cane or molasses, cassava and sap from the Rafia palm can be processed, fermented and distilled to obtain spirits. Although the major physiologically active component of most alcoholic beverages is ethyl alcohol (ethanol), there is also a remaining fraction of highly volatile compounds called congeners [2].

Congeners are impurities in alcoholic beverages other than the desired type of alcohol (ethanol), produced during fermentation. They are responsible for the taste, aroma and colour in alcoholic beverages. They are produced in the process of fermentation or ageing, when organic compounds in the beverage break down. These include acetaldehydes, esters, ethyl esters, carbonyl compounds and acid. Congener content of commercial alcoholic beverages differs significantly for each type of beverage, wine and beer having appreciably higher amounts than distilled spirits. The level of these compounds are influenced by several processing factors such as type/source of raw material, fermentation condition and distillation techniques; and all these factors define the chemical quality of the spirit. Amongst these chemical compounds, the main compounds considered to be contaminants in spirits are ethyl carbamate, methanol among others.

Ethyl carbamate (EC) or urethane is the ethyl ester of carbamic acid. It can be found in fermented foods and beverages like spirits, wine, beers, bread and yoghurt [3]. It can be formed by various substances such as hydrocyanic acid or through reaction between urea and ethanol during yeast fermentation as well as by heating or long periods of storage [4]. Once EC is formed, it is chemically stable and cannot be easily decomposed [4,5]. Its formation as a contaminant was linked in 1971 with antimicrobial agent, diethyldicarbonate (DEDC) and 2.6 ppm ethyl carbamate was reported in wine [6]. WHO [7] classified EC as toxic by the European Union and is regarded as probably carcinogenic to humans. The Scientific Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA) concluded that ethyl carbamate in alcoholic beverages indicates a health concern, particularly with respect to alcoholic beverages. EFSA recommended taking mitigation measures to reduce the levels of ethyl carbamate in these beverages [8].

However, the prevalence of congeners especially ethyl carbamate in spirit beverages from locally available raw materials is yet to be evaluated as there is little or no information on congeners from spirits produced from cassava, palm wine and molasses fermentates. There is also no known standard in the mean time for the levels of ethyl carbamate and other contaminants in spirit beverages produced in Nigeria. These alcoholic beverages are locally produced, readily available, cheap, and are mostly consumed by low-income earners in preference to the more expensive brewed beer, spirit, and rums, etc [9]. However, the consumption of these drink types is not restricted to low-income earners alone, higher income earners also consume these drinks. These drink types are the favourite drinks for traditional occasions in most West African countries [9]. Therefore, to satisfy official food control regulations, it is essential to determine whether there is presence of ethyl carbamate and other possible contaminants in the distillates of fermented products from cassava, molasses and palm wine.

A good understanding on the EC level and physicochemical properties of spirits from fermented mashes of cassava, molasses and palm wine is important for guiding researchers and manufacturers on processing conditions for optimized output, thus improve the quality of production of alcoholic beverages (spirits). This research work will create awareness on the presence of congeners in locally produced spirits and other alcoholic beverages, and thus be a guide for regulatory agencies in Nigeria to set standards and acceptable levels of congeners and other possible contaminants such as ethyl carbamate and ethyl acetate for locally distilled and blended alcoholic beverages.

2. MATERIALS AND METHODS

2.1 Sample Procurement

Cassava tubers (*Manihot esculenta*) were obtained from Akokwa in Imo State, Obosi in Anambra State and Abakiliki in Ebonyi State. Palm wine was obtained from Izombe in Imo State, Nsugbe in Anambra State, Bende in Abia State, Ikom in Cross River State and Nimbo in Enugu State. Molasses from the following sugar cane varieties (SP71/6180, B447-419 and C0957) were collected from Nigerian sugar factory, Bacita in Kogi State.

The processing of samples and experiments were carried out using the facilities available at the laboratories of Department of Food Science and Technology, Federal University of Technology, Owerri; Project Development Agency (PRODA) Enugu and International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State.

2.2 Sources of Enzymes and Yeast

Industrial enzymes (α-amylase and β-amylase) were obtained from Nigerian Breweries Plc, Awo-nmanma while the wine yeast (*Saccharomyces cerevisiae var cerevisiae*) was supplied by Bioferm Rouge, Beverlo, Belgium.

2.3 Sample preparation

2.3.1 Extraction of starch from cassava tubers

The Starch extraction of cassava tubers were carried out according to the methods described by Osuji and Anih [10]. The tubers were washed, peeled and wet milled into slurry. The slurry was stirred and allowed to settle for about 6 hours. A heterogeneous mixture was observed. The top part of it was a transparent liquid while the bottom part was a thick liquid (the starch). The supernatant was decanted and the sediment filtered with muslin cloth and oven-dried at 70˚C for 30 minutes to produce the dry starch.

2.3.2 Production of Spirit from Dry cassava starch

The dry cassava starch was reconstituted into slurry, homogenized using a spindle in a beaker on a thermostatically-controlled heating mantle at 80°C, until the mixture was gelatinized. This was followed by enzyme hydrolysis in which the medium was cooled to 40°C and then 50ml α-amylase added. The medium was allowed to stand for 2 hours and liquefaction achieved. β-amylase (50ml) was added and the medium allowed to stand for another 2 hours saccharification achieved. The resultant starch hydrolysates were boiled to denature the enzymes, then cooled in an ice water bath. The hydrolysates were fermented with wine yeast for 4 days at 24°C±1°C. After fermentation, the liquor was distilled at 78.5°C and the distillate (spirit) obtained was packaged in an air-tight container.

2.3.3 Production of spirit from Molasses

Molasses collected from sugar cane of different varieties were diluted into four folds up to 8-10° brix and fermented with wine yeast for 4 days at 24°C±1°C. After fermentation, the liquor was distilled at 78.5°C and the distillate (spirit) obtained was packaged in an air-tight container.

2.3.4 Production of spirit from palm wine

Palm wine was allowed to ferment for 2 days. The product was distilled and the distillate packaged in an air tight container.

2.4 Description of samples for analysis

The samples obtained from cassava, molasses and palm wine were labelled in the following order:

Code	Sample Description and location
CA1	Cassava tubers from Akokwa, Imo State
CA2	Cassava tubers from Warri, Delta State
CA3	Cassava tubers from Obosi, Anambra State
CA4	Cassava tubers from Abakiliki, Ebonyi State
PW1	Palm wine from Izombe, Imo State
PW2	Palm wine from Nsugbe, Anambra State
PW3	Palm wine from Bende, Abia State
PW4	Palm wine from Ikom, Cross Rivers State
PW5	Palm wine from Nimbo, Enugu State.
MS1	Molasses from
MS2	Molasses from Sugar cane variety Bacita SP71/6180 in Kwara State
MS3	Molasses from Sugar cane variety Bacita B447-419 in Kwara State
MS4	Molasses from Sugar cane variety Bacita C0957 in Kwara State

2.5 Quality Evaluation of distillates from Cassava, palm wine and sugar cane fermentates.

2.5.1 Determination of Ethyl Carbamate

Ethyl carbamate was determined using a Gas Chromatograph (G.C), Model SRI 8610 (FID/ECD) according to the method described by AOAC [11].

2.5.2 pH Determination

The distillate sample pH was determined using AOAC [12] procedure.

2.5.3 Determination of Viscosity

The viscosity was determined according the method described by Ademiluyi and Mepba [13].

2.5.4 Determination of Specific Gravity

The specific gravity was determined according the method described by Ademiluyi and Mepba [13].

2.5.5 Determination of Alcohols and Esters

Alcoholic compounds (ethanol, propanol, isobutyl alcohol, hexanol, etc) and esters (ethyl hexanoate, ethyl octanoate, ethyl decanoate, etc) were determined using a Gas Chromatograph (G.C), Model SRI 8610 (FID/ECD) according to the method described by AOAC [11].

2.6 Statistical Analysis

Data obtained from this experiment was subjected to One Way Analysis of Variance (ANOVA) classification using SPSS 16 Software Package [14].

3. RESULTS AND DISCUSSION

3.1 PHYSICOCHEMICAL PROPERTIES OF DISTILLATES FROM CASSAVA, MOLASSES AND PALM WINE.

The mean values of pH, viscosity and specific gravity (SG) of distillates (spirits) from molasses, cassava and palm wine from different locations are summarized in Table 1.

3.1.1 pH:

The pH of the entire distillate ranged from 3.40-5.30 and all the spirit beverages were significantly different ($P = .05$) from each other. The distillate from MS1 (Molasses) recorded the highest pH with a mean value of 5.30 followed by CA4 (Cassava from Abakiliki) with a mean value pH of 5.20 which are not significantly different ($P > .05$), while the distillate from CA1 (Cassava from Akokwa) recorded the least pH with a mean value of 3.40. The variations in pH could be attributed to variations in source of raw materials and the harvest locations. The pH of the distillates was in acid region. pH is one of the main quality characteristics that describes the stability of bioactive compounds in food products [15]. It is also the negative logarithm of hydrogen ion concentration in a solution [16]. An acidic pH is related to the presence of organic acids in the spirits [17]. In addition, pH can also be dependent on oxidation reactions of some distillates' constituents in contact with some mineral ion like calcium [18]. When comparing the pH of these distillates (spirits) with other spirit beverages, it is observed that these distillates presents a lower pH than Agave sap type 1 (6.6–7.5) [19], presumably because some of the volatile components that predominate in the former are carboxylic acids that evidently contribute to the acidity of the beverage. Other components added along the production process might be responsible for the acidity [20,21] particularly the addition and use of *Saccharomyces cerevisiae* during the fermentation process [20].

3.1.2 Specific Gravity:

The mean values of specific gravity (SG) of the distillates ranged from 0.9700 to 1.1200 with an average of 1.208 and there were no significant differences ($P > .05$) amongst the distillates. However, Carreon-Alvarez *et al.*, [22] reported that the specific gravity of some spirit beverages from Africa including Burukutu, Palm wine, Ogogoro, among others ranged from 0.9897 to 1.318 with an average of 1.1015. The specific gravity of the spirit is the density of the spirits from different sources compared to that of water at equal volumes [23].

The variations in the specific gravity of these spirits might have been due to principally varying levels of alcohol contents and other constituents such as congeners in the spirits which contributed to its density.

3.1.3 Viscosity:

Viscosity is a highly relevant parameter; it determines the acceptability, processing, and handling of foods [24]. The mean values of viscosity of spirits obtained from palm wine, cassava and molasses distillates from different locations ranged from 7.402mPa.s to 9.937mPa.s with an average viscosity of 8.831mPa.s. There were no significant differences ($P > .05$) amongst the viscosity of the resultant spirits. The viscosity of spirits obtained in this study is slightly higher than the average viscosity of silver tequilas (2.48mPa·s) reported by Carreon-Alvarez *et al.*, [22]. Since viscosity is closely related to the concentration of molecular components in a beverage including ethanol content, it is also relevant for spirit beverages. A higher viscosity of the obtained spirits when compared to tequila and previous works could be an indication that a combination of varying concentrations of congeners such as ethyl carbamate, esters and alcohol are present. In addition, viscosity depends on molecules with large molecular weights, molecular structure, and hydrogen bridges between OH– and water [25]. As above mentioned, these differences are likely due to presence of compounds with different molecular weights. In comparison to the average viscosity (8.831mPa.s) of spirit obtained in this present study, the viscosity of the distilled beverage is about 0.9918mPa·s and 1.24mPa·s for ethyl alcohol [22] similar to values reported in the literature: 0.815mPa·s and 0.964mPa·s, respectively [26]. Therefore, it is evident that the presence of congeners in high concentrations could be responsible for the higher viscosity of spirits from palm wine, cassava and molasses obtained from different locations. Generally, liquids with low molecular weight tend to behave as Newtonian fluids, whereas polymers with high molecular weight are usually non-Newtonian. According to the obtained results, the resultant spirits usually behaves as Newtonian-like fluid [24].

3.1.4 Ethyl Carbamate (EC) Content:

The mean values of ethyl carbamate content ranged from 11.95µg/l to 15.11µg/l with the highest EC content (15.11µg/l) recorded for PW4 (Palm wine from Ikom) while MS1 (Molasses) with a mean value of 11.95µg/l having the least EC content. There were significant differences (*P = .05*) amongst the ethyl carbamate content of the various spirits from different locations.

From the results, the EC content of distillates of cassava, palm wine and molasses recorded an average of 13.44µg/l, 13.75µg/l and 12.49µg/l respectively. Levels of average ethyl carbamate in the spirits were found to follow this order PW (13.75 µg/l)> CA (13.44 µg/l) > MS (12.49 µg/l) though there was no significant difference (*P > .05*) between average EC contents obtained from different locations. However, these levels of EC content were lower than the maximum permissible level of NAFDAC (1.50mg/l) [27] and the Canadian limit of 0.15mg/l [28] for distilled spirits as well as the maximum allowed EC level of distilled spirits in USA, Czech Republic and France to be 150µg/l as reported by Alexander *et al.,* [29]. The observable lower EC content of distilled spirits from cassava, palm wine and molasses from different locations could be attributed to the reduced amounts of EC precursors in the raw materials (cassava, molasses and palm wine) [30]. It could also be due to the complete metabolism of nitrogen-containing compounds during the fermentation process [31].

Ethyl carbamate is a compound that can occur naturally in fermented foods and beverages, such as spirits, wine, beer, bread, soy sauce and yoghurt. Therefore, the major source of dietary exposure to ethyl carbamate in the human population is through the consumption of fermented foods and beverages, e.g. as a consequence of its unintentional formation during the fermentation process or during storage [29]. Ethyl carbamate can be formed from various substances derived from food and beverages, including hydrogen cyanide, urea, citrulline, and other N-carbamyl compounds. Cyanate is probably the ultimate precursor in most cases, reacting with ethanol to form the carbamate ester.

Table 1: Physicochemical Properties of Distillates from Cassava, Molasses and Palm Wine

SAMPLE	pH	Viscosity (mPa.s)	SG	Ethyl Carbamate (µg/l)
CA1	$3.40^{c}\pm0.01$	9.937±0.00	1.02±0.01	12.95±0.12
CA2	$4.70^{b}\pm0.01$	8.752±0.01	0.98±0.01	13.26±0.25
CA3	$4.40^{b}\pm0.01$	9.637±0.01	1.02±0.02	13.96±0.20
CA4	$5.20^{a}\pm0.02$	7.402±0.02	1.12±0.02	13.60±0.22
PW1	$3.80^{c}\pm0.02$	7.601±0.01	1.00±0.01	12.64±0.10
PW2	$4.60^{b}\pm0.01$	9.351±0.02	0.97±0.01	12.90±0.23
PW3	$5.90^{a}\pm0.01$	8.483±0.01	0.99±0.01	13.86±0.22
PW4	$3.70^{c}\pm0.01$	8.589±0.02	1.01±0.02	15.11±0.12
PW5	$3.90^{b}\pm0.02$	9.197±0.01	0.98±0.01	14.26±0.11
MS1	$5.30^{a}\pm0.01$	9.487±0.01	1.12±0.02	11.95±0.23
MS2	$4.50^{b}\pm0.02$	8.438±0.02	1.09±0.01	12.95±0.22
MS3	$3.70^{c}\pm0.01$	7.987±0.01	1.00±0.01	12.21±0.23
MS4	$4.70^{b}\pm0.02$	9.936±0.01	0.97±0.01	12.86±0.11
LSD	**0.12**	**NS**	**NS**	

Values are the means of duplicate determinations

a,b….means with the same superscript along a column is not significantly different ($P > .05$).

KEYS:

CA1 = Cassava from Akokwa

CA2 = Cassava from Warri

CA3 = Cassava from Obosi

CA4 = Cassava from Abakiliki

PW1 = Palm wine from Izombe

PW2 = Palm wine from Nsugbe

PW3 =Palm wine from Bende

PW4 = Palm wine from Ikom

PW5 = Palm wine from Nimbo

MS1 = Molasses

MS2 = Molasses of Sugar cane from Bacita SP71/6180

MS3 = Molasses of Sugar cane from B447-419

MS4 = Molasses of Sugar cane from C0957

3.2 Mean Values of Alcohols from Distillates of Cassava, Molasses and Palm Wine

The mean values of alcohol content of distillates (spirits) from molasses, cassava and palm wine from different locations are summarized in Table 2 and Table 3.

3.2.1 Ethanol:

The mean values of ethanol content ranged from 36.14% to 39.12% with the highest ethanol content (39.12%) recorded for PW4 (Palm wine from Ikom) while palm wine from Izombe with a mean value of 36.14% having the least ethanol content. There were significant differences ($P = .05$) amongst the ethanol contents of the various spirits from different locations.

From the results, the ethanol content of distillates of cassava, palm wine and molasses recorded an average of 37.67%, 37.79% and 37.50% respectively. Levels of ethanol content in the spirits were found to follow this order PW (37.79%)> CA (37.67%) > MS (37.50%) though there was no significant difference ($P > .05$) between the average ethanol contents obtained from different locations. Ethanol is present in alcoholic beverages as a consequence of the fermentation of carbohydrates with yeast and it's responsible for the beverage's body [32]. It is produced by yeasts through the glycolytic pathways, with pyruvate being the key intermediate compound, which is then decarboxylated into acetaldehyde, followed by reduction to ethanol.

The ethanol concentration in different types of spirits is defined by International Regulation [33]. Its determination is part of the quality control of spirit drinks. Following this regulation, the distilled spirits made from cassava, molasses and palm wine fermentates met the minimum limit approved for the ethanol concentration which is set from 36% to 37.5% (V/V). In all tested samples, the mean values of the ethanol content which ranged from 36.14% to 39.12% was found to be in compliance with the proposed Regulations [33]. The significant high ethanol concentration of spirits obtained could be as a result of the sugar profile and its concentrations, the type and nature of yeast strain inoculated during fermentation and the quantity of mash and fermentate distilled to obtain the various spirit.

As noted previously, ethanol not only gives off an alcoholic odour and body to beverages, but also acts as a carrier of other odour-active volatile compounds [34]. Ethyl alcohol is the only alcohol generally present in sufficient amount to be of sensory significance in spirits. It generates a complex of sensory perceptions as it possesses a distinctive odour, activates the perception of sweetness, and stimulates the sensations of heat and weight in the mouth. Ethanol can also mask or modify other spirit sensations (Jackson, 2017). However, these complex effects and perceptions are influenced by the concentration of ethanol in the spirits. In this study, the high concentrations of ethanol content from cassava, molasses and palm wine distillates could affect the sensory perception of the product.

3.2.2 Higher Alcohols:

Aside ethanol, higher alcohols (also known as fusel alcohols or fusel oils) are the major alcohols that impart sensory properties to spirits. In this study, the higher alcohols obtained from distilled spirits of cassava, molasses and palm wine from different locations includes ethyl ether, 1-propanol, isobutyl alcohol, isoamyl alcohol, 1-hexanol, phenethyl alcohol and pentyldecanol. The mean values of these components of higher alcohols are shown in Table 2 and Table 3. The mean values of ethyl ether ranged from 0.26% to 0.86%, 1-propanol ranged from 0.05% to 0.13% while isobutyl alcohol, ranged from 0.02% to 0.09%. There were no significant differences ($P > .05$) in spirits of cassava, molasses and palm wine for ethyl ether, 1-propanol and isobutyl alcohol. The mean values of isoamyl alcohol ranged from 0.12% to 0.76%, 1-hexanol ranged from 0.12% to 0.46%, phenethyl alcohol ranged from 0.24% to 0.82% while pentyldecanol ranged from 0.47% to 1.63%. There were significant differences ($P = .05$) in spirits of cassava, molasses and palm wine for 1-hexanol, phenethyl alcohol, pentyldecanol and isoamyl alcohol. The concentrations of the identified higher alcohols in the various spirits were relatively low compared to the concentration of ethanol contents of spirits obtained in this research. The combination of ethanol content and higher alcohols makes up the total alcohol which ranged from 39.00% to 46.71%. The high alcohol content of spirits from cassava, palm wine and molasses from this study could be attributable to the nature of raw material, high sugar concentration in the mash to yield enough ethanol and other fermentation by-products, good fermentation conditions and quantity of fermentates.

Higher alcohols or fusel alcohols are quantitatively major volatile by-products of fermentation and are thought to contribute to the aromatic complexity of spirit [35]. Higher alcohols can be biosynthesized by yeasts from sugars and selected amino acids (typically branched-chain and aromatic amino acids) via the anabolic pathway and Ehrlich pathway, respectively [36]. In addition, higher alcohols impart a range of organoleptic attributes such as alcoholic, fruity, pungent, solvent-like and rose-like or floral, depending on the concentration and type of alcohol [36]. The aroma importance of higher alcohols extends to other facets of spirit flavour by serving as ester precursors. Higher concentrations negatively impact on spirit aroma by contributing harsh aroma and taste. They also appear to play a role in varietal character [37,38].

Although ethanol has a mild fragrance, the most significant aromatic alcohols are the higher (fusel) alcohols, those with carbon chains three to six carbons long. Examples such as 1-propanol, isobutyl alcohol, 2-methyl-1-butanol, and isoamyl alcohol tend to have fusel odors, whereas hexanols possess a herbaceous scent. The major phenol-derived alcohol, 2-phenylethanol (phenethyl alcohol), has a rose-like scent [34]. In food legislation, the content of higher alcohols in alcoholic beverages is generally not seen as toxicologically relevant. For example, the Joint FAO/WHO Expert Committee on Food Additives included some higher alcohols like 1-propanol, 1-butanol and isobutanol in the functional class 'flavouring agent' and commented that there was no safety concern at current levels of intake [39]. For certain groups of spirits, the European Union even demands a minimum volatile substance content (i.e., the quantity of volatile substances, mainly higher alcohols, other than ethanol and methanol). The presence of these higher alcohols though in low concentrations is evident that the spirits from cassava, molasses and palm wine would impart some aromatic properties in the final product.

Table 2: Mean Values of Ethanol, Ethyl Ether, 1-Propanol, Isobutyl Alcohol and Isoamyl Alcohol

SAMPLE	Ethanol (%)	Ethyl ether (%)	1-Propanol (%)	Isobutyl alcohol (%)	Isoamyl alcohol (%)
CA1	$37.05^{bc}\pm0.01$	0.26±0.01	0.08±0.01	0.07±0.01	$0.66^{c}\pm0.03$
CA2	$38.60^{ab}\pm0.01$	0.37±0.01	0.07±0.01	0.04±0.01	$0.73^{b}\pm0.20$
CA3	$38.20^{ab}\pm0.03$	0.63±0.01	0.07±0.01	0.09±0.01	$0.60^{d}\pm0.03$
CA4	$36.84^{c}\pm0.01$	0.82±0.01	0.05±0.01	0.06±0.01	$0.39^{h}\pm0.01$
PW1	$36.14^{c}\pm0.02$	0.40±0.01	0.07±0.01	0.03±0.00	$0.50^{f}\pm0.01$
PW2	$36.22^{c}\pm0.02$	0.43±0.01	0.08±0.01	0.06±0.00	$0.34^{h}\pm0.03$
PW3	$38.63^{ab}\pm0.01$	0.86±0.01	0.09±0.01	0.09±0.01	$0.12^{i}\pm0.03$
PW4	$39.12^{a}\pm0.02$	0.65±0.02	0.06±0.01	0.03±0.00	$0.38^{h}\pm0.03$
PW5	$38.82^{ab}\pm0.03$	0.32±0.01	0.13±0.01	0.02±0.01	$0.76^{a}\pm0.01$
MS1	$37.02^{bc}\pm0.01$	0.31±0.01	0.09±0.01	0.05±0.01	$0.59^{e}\pm0.01$
MS2	$37.50^{bc}\pm0.01$	0.52±0.01	0.10±0.01	0.09±0.00	$0.44^{g}\pm0.03$
MS3	$37.60^{bc}\pm0.02$	0.32±0.01	0.09±0.01	0.04±0.01	$0.59^{e}\pm0.01$
MS4	$37.87^{bc}\pm0.02$	0.43±0.01	0.11±0.01	0.07±0.00	$0.44^{g}\pm0.01$
LSD	**1.80**	**NS**	**NS**	**NS**	**0.06**

Values are the means of duplicate determinations

a,b….means with the same superscript along a column is not significantly different (*P > .05*).

KEYS:

CA1 = Cassava from Akokwa

CA2 = Cassava from Warri

CA3 = Cassava from Obosi

CA4 = Cassava from Abakiliki

PW1 = Palm wine from Izombe

PW2 = Palm wine from Nsugbe

PW3 =Palm wine from Bende

PW4 = Palm wine from Ikom

PW5 = Palm wine from Nimbo

MS1 = Molasses

MS2 = Molasses of Sugar cane from Bacita SP71/6180

MS3 = Molasses of Sugar cane from B447-419

MS4 = Molasses of Sugar cane from C0957

Table 3: Mean Values of Hexan-1-ol, Phenethyl Alcohol, Pentyldecanol and Total Alcohol

SAMPLE	Hexan-1-ol (%)	Phenethyl alcohol (%)	Pentyldecanol (%)	Total alcohol (%)
CA1	0.33^{b}±0.01	0.73^{a}±0.01	0.86^{e}±0.01	42.45^{b}±0.32
CA2	0.46^{a}±0.01	0.85^{a}±0.02	0.88^{e}±0.01	45.19^{a}±0.04
CA3	0.39^{ab}±0.01	0.82^{a}±0.02	1.10^{c}±0.04	46.71^{a}±0.04
CA4	0.12^{d}±0.01	0.46^{a}±0.01	1.30^{b}±0.01	39.67^{d}±0.32
PW1	0.17^{d}±0.01	0.55^{a}±0.01	1.12^{c}±0.02	40.49^{cd}±0.04
PW2	0.23^{c}±0.01	0.64^{a}±0.01	1.63^{a}±0.01	40.13^{cd}±0.02
PW3	0.16^{d}±0.01	0.38^{a}±0.01	1.16^{c}±0.01	41.07^{bc}±0.32
PW4	0.15^{d}±0.01	0.38^{a}±0.01	0.99^{d}±0.02	41.46^{bc}±0.04
PW5	0.17^{d}±0.01	0.60^{a}±0.02	0.65^{f}±0.01	40.82^{cd}±0.02
MS1	0.18^{d}±0.01	0.47^{a}±0.01	0.53^{g}±0.01	39.06^{d}±0.32
MS2	0.15^{d}±0.01	0.38^{a}±0.02	0.85^{e}±0.02	39.02^{d}±0.04
MS3	0.14^{d}±0.01	0.24^{b}±0.01	0.62^{f}±0.01	39.56^{d}±0.32
MS4	0.14^{d}±0.01	0.24^{b}±0.01	0.47^{h}±0.02	39.00^{d}±0.04
LSD	**0.09**	**0.77**	**0.09**	**1.80**

Values are the means of duplicate determinations

a,b….means with the same superscript along a column is not significantly different (*P > .05*).

KEYS:

CA1 = Cassava from Akokwa

CA2 = Cassava from Warri

CA3 = Cassava from Obosi

CA4 = Cassava from Abakiliki

PW1 = Palm wine from Izombe

PW2 = Palm wine from Nsugbe

PW3 =Palm wine from Bende

PW4 = Palm wine from Ikom

PW5 = Palm wine from Nimbo

MS1 = Molasses

MS2 = Molasses of Sugar cane from Bacita SP71/6180

MS3 = Molasses of Sugar cane from B447-419

MS4 = Molasses of Sugar cane from C0957

3.3 Mean Values of Esters

The mean values of esters of distillates (spirits) from molasses, cassava and palm wine from different locations are summarized in Table 4.

<u>3.3.1 Esters:</u>

The mean values of esters from spirits of cassava, molasses and palm wine from different locations is shown in Table 4.. The distilled spirits yielded a combination of esters such as ethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and phenethyl butanoate in various proportions. The mean values of ethyl acetate, ethyl hexanote, ethyl octanoate, ethyl decanoate, phenethyle butanoate and dimethyl styrene ranged from 1.20 mg/l to 5.06 mg/l, 0.07mg/l to 0.29 mg/l, 0.08 mg/l to 0.09 mg/l, 0.17mg/l to 0.66mg/l and 0.13 mg/l to 0.51 mg/l respectively. There were significant differences ($P = .05$) in spirits of cassava, molasses and palm wine for all the esters identified except for ethyl octanoate. The ester profile identified in this study is similar to that reported by Nwaiwu *et al.* [40].

Esters are very important compounds due to their particular contribution to flavour and aroma, since they have the lowest organoleptic threshold [41]. The quantity of this compound presented in the final product can vary widely, since it is synthesized from acetic acid and ethanol [42]. They are formed during alcoholic fermentation via yeast metabolism and qualitatively present the major class of flavour compounds in distillates [43].

Ethyl acetate is the major ester present in alcoholic beverages. Esters, generally, are associated with a pleasant, fruity and flowery aroma. Their contribution to flavour is strongly influenced by their concentration [44, 45]. As can be seen in Table 4., ethyl acetate was the dominant ester, as expected. In very small quantities, ethyl acetate contributes to the pleasant smell of distillates. Concerning ethyl acetate, many authors have documented high variability [44, 46]. The average mean values of the concentration of ethyl acetate for spirit of cassava was 2.37mg/l, 3.11mg/l for spirit of palm wine and 1.91mg/l for spirit of molasses. The results obtained by Winterova *et al*., [47] for the content of ethyl acetate in plum brandy were significantly ($P = .05$) higher than the ethyl acetate content found in the present study. Also, Madrera *et al*., [48] reported an ethyl acetate content in the range of 20.45mg/l to 2045mg/l in a cider spirit. Though ethyl acetate is regarded as a volatile congener and possibly a contaminant according to Alcarde *et al*., [49] and Osobamiro [50], comparing the results obtained, it could be concluded that the concentrations of ethyl acetate found in spirits of cassava, molasses and palm wine are commonly acceptable.

According to Madrera *et al*., [48], the ethyl hexanoate content identified in cider spirit was 0.30mg/l to 14.7mg/l, 0.30mg/l to 15.6mg/l for ethyl octanoate, 0.30mg/l to 15.9mg/l for ethyl decanoate while 0.11mg/l to 4.20mg/l was recorded for phenethyl butanoate. It is evident that esters obtained in the present study is significantly ($P = .05$) lower when compared to those obtained by Madrera *et al.,* [48]. The observable decrease in esters could be as a result of proper and favourable fermentation which led to low acetic acid production.

Table 4: Esters of Distillates from Cassava, Molasses and Palm Wine

SAMPLE	Ethyl acetate (mg/l)	Ethyl hexanoate (mg/l)	Ethyl octanoate (mg/l)	Ethyl decanoate (mg/l)	Phenethyl butanoate (mg/l)	Total esters (mg/l)
CA1	$3.29^{d}\pm0.02$	$0.18^{b}\pm0.01$	0.08 ± 0.01	$0.45^{d}\pm0.01$	$0.33^{b}\pm0.02$	$4.54^{c}\pm0.04$
CA2	$3.26^{d}\pm0.01$	$0.20^{b}\pm0.01$	0.08 ± 0.01	$0.36^{g}\pm0.01$	$0.30^{d}\pm0.01$	$4.32^{d}\pm0.04$
CA3	$1.57^{h}\pm0.02$	$0.14^{cd}\pm0.02$	0.08 ± 0.01	$0.66^{a}\pm0.02$	$0.23^{e}\pm0.02$	$2.83^{f}\pm0.02$
CA4	$1.37^{i}\pm0.04$	$0.12^{c}\pm0.02$	0.09 ± 0.01	$0.54^{c}\pm0.02$	$0.24^{e}\pm0.02$	$2.62^{g}\pm0.04$
PW1	$3.96^{b}\pm0.04$	$0.23^{ab}\pm0.02$	0.08 ± 0.01	$0.63^{b}\pm0.01$	$0.24^{e}\pm0.01$	$5.29^{b}\pm0.04$
PW2	$2.91^{e}\pm0.04$	$0.29^{a}\pm0.02$	0.09 ± 0.01	$0.36^{g}\pm0.02$	$0.35^{b}\pm0.02$	$4.19^{e}\pm0.04$
PW3	$5.06^{a}\pm0.04$	$0.17^{b}\pm0.01$	0.08 ± 0.01	$0.51^{e}\pm0.02$	$0.51^{a}\pm0.01$	$6.85^{a}\pm0.04$
PW4	$1.66^{g}\pm0.04$	$0.22^{b}\pm0.02$	0.08 ± 0.01	$0.29^{h}\pm0.02$	$0.20^{f}\pm0.02$	$2.58^{g}\pm0.04$
PW5	$1.98^{f}\pm0.04$	$0.08^{e}\pm0.01$	0.09 ± 0.01	$0.17^{j}\pm0.02$	$0.36^{b}\pm0.01$	$2.89^{f}\pm0.03$
MS1	$3.58^{c}\pm0.04$	$0.08^{e}\pm0.01$	0.08 ± 0.01	$0.18^{ij}\pm0.01$	$0.32^{c}\pm0.01$	$4.46^{c}\pm0.04$
MS2	$1.33^{i}\pm0.04$	$0.07^{e}\pm0.01$	0.09 ± 0.01	$0.38^{f}\pm0.01$	$0.32^{c}\pm0.01$	$2.83^{f}\pm0.03$
MS3	$1.51^{h}\pm0.04$	$0.10^{d}\pm0.01$	0.08 ± 0.01	$0.19^{i}\pm0.01$	$0.24^{e}\pm0.01$	$2.38^{h}\pm0.03$
MS4	$1.20^{f}\pm0.02$	$0.06^{e}\pm0.01$	0.08 ± 0.01	$0.14^{k}\pm0.01$	$0.13^{f}\pm0.04$	$0.78^{i}\pm0.04$
LSD	**0.06**	**0.06**	**NS**	**0.01**	**0.02**	**0.09**

Values are the means of duplicate determinations

a,b….means with the same superscript along a column is not significantly different (*P > .05*).

KEYS:

CA1 = Cassava from Akokwa

CA2 = Cassava from Warri

CA3 = Cassava from Obosi

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MS1 = Molasses

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MS3 = Molasses of Sugar cane from B447-419

MS4 = Molasses of Sugar cane from C0957

4. Conclusion

The distillation of a cassava, palm wine and molasses derived fermented liquor resulted in a product that is basically composed of alcohols and congeners such as ethyl carbamate, aldehydes and esters. The distilled spirits presented some aversive attributes that give spirits its aromatic characteristic quality due to availability of some volatile compounds that can be attenuated by aging. Ethyl carbamate and ethyl acetate which are considered contaminants were significantly below the permissible level of NAFDAC and Canadian limits for distilled spirits. The good fermentation employed in this research work significantly reduced the concentrations of the detected congeners.

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