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Comparative Studies of the Physicochemical Properties of Beers Brewed with Hop Extracts and Extracts from Four Selected Tropical Plants

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ABSTRACT

Hops, a minor ingredient in beer, are used for their bittering, flavouring and aroma enhancing powers. Their pronounced bacteriostatic activity inhibits the growth of gram-positive bacteria in the finished beer thereby extending the shelf life of the product. They are grown throughout the temperate regions of the world. The potential of four selected tropical plants: *Azadirachta indica* (neem), *Garcinia kola* (bitter kola), *Gongronema latifolium* (heckel) and *Vernonia amygdalina* (bitter leaf) as substitutes for hops in beer brewing were evaluated. The ethanolic extracts of the plant parts commonly consumed by people were used to brew beers. The physicochemical properties of the finished beer products were studied using standard methods. These tropical plants were statistically ranked by the application of Analysis of Variance (ANOVA) using beers brewed with hop extracts as controls. The physicochemical properties of the brewed beers revealed that the alcohol content in all the beer samples ranged between 3.43-3.75%, total acidity from 0.132-0.324%, pH (5.47-5.68), turbidity (5-125 NTU), total solids ranged from 3.66-8.16% and bitterness level ranged from 25.38-39.62 IBU. The concentration of arsenic in the beer samples ranged from 1.44-1.77 ppm while that of metals were cadmium (0.00-0.97 ppm) and copper (0.10-2.70 ppm). It was established from ranking that the order of closeness of the vegetables investigated to isomerized hop extract was *G. kola* (0.969) > *G. latifolium* (0.609) > *V. amygdalina* (0.601) > *A. indica* (0.536) while that to hop leaf extract was *G. kola* (0.964) > *A. indica* (0.800) > *G. latifolium* (0.440) > *V. amygdalina* (0.433).

1. Introduction

Beer is defined as an alcoholic drink made from yeast fermented malt, flavored with hops. Beer production worldwide is a viable industry. Among commercial beverages in 2006, beer ranks fourth in per capita consumption behind carbonated soft drinks, bottled water and coffee followed by milk and fruit drinks in the United States of America. Per capita beer consumption rose rapidly during the second world-war, declined during the 1950s and early 1960s, increased before peaking in the early 1980s and has generally leveled-off thereafter [1].

A similar trend is reported of the beer industry in Nigeria by Badmus [2] who observed that the Nigerian beer industry is a very vital component of Nigeria's non-oil sector and has largely contributed to economic growth in recent times. This can be attributed to the country's favorable demographics with populous and vibrant youth and growing middle class. This, along with a growing, largely youth population with increased disposable incomes is the constant drive that increased beer consumption in Nigeria.

From medieval times, herbs have been used to flavor and preserve fermented malt liquors but only hop inflorescence is used on a commercial scale today [3]. Hop plants are vital to the brewing industry and some of their unique chemicals have the potential to be used in the nutraceutical industry [4]. Hop extracts give beer its bitter taste, improve foam stability, enhance aroma and flavor and act as antiseptic towards microorganisms [5]. Hop plants are grown throughout the temperate regions of the world. Nigeria is in tropical region and since beer production in Nigeria has never declined with ready market as consumption rate continues to increase, the importation of hops becomes inevitable. Thus, the need to investigate some Nigerian plants that could substitute hops in beer brewing.

Some pioneer work showed that leaves of the vegetable, *Gongronema latifolium* (*utazi*) have great potential as substitute for hops in tropical beer brewing. It was found out that this plant possessed some antiseptic properties against beer spoilage microorganisms. The chemical properties of beer brewed using this plant did not differ much from that brewed with hops though their organoleptic differences were pronounced [6]. The authors however did not characterize the vegetables as they only used it for brewing and sensory analysis. Ajebesone and Aina [7] carried out proximate analysis of four tropical plants used for food in Africa namely *Azadirachta indica* (neem), *Garcinia kola* (bitter kola), *Gongronema latifolium* (heckel) and *Vernonia amygdalina* (bitter leaf). Those authors concluded that these tropical vegetables can serve as substitutes for hops in tropical beer brewing. The use of bitter leaf as substitute for hops in the Nigerian brewing industry has been chronicled by Adama et al. *Azadirachta indica* is used in some parts of Nigeria for treatment of malaria while *Garcinia kola* is used in some areas for the treatment of stomach ache and gastritis. *Vernonia amygdalina* and *Gongronema latifolium* are widely consumed as vegetables [8-10]. One thing common to all the four plants is that they are bitter, like hops, but thrive in tropical regions, unlike hops [7].

The aim of this study was to investigate the potential of the extracts of four selected tropical plants (bitter kola, neem, bitter leaf and heckel) as local and available hop substitutes in the production of beer.

If it is categorically established in this study that these tropical plants can substitute hops in the production of premium quality and world class beers, the brewing industries in the tropical regions of the World will no longer depend on imported hops. This level of raw material freedom confers definite economic advantages to the Nigerian brewing industry.

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2. Experimental Methods

2.1 Procurement of Materials

Hop leaf and isomerised hop extract were respectively purchased from Youngs Ubrew Goldings Hops and Ritchies both in the United Kingdom. The leaves of *A. indica*, *G. latifolium*, *V. amygdalina* and the seeds of *G. kola* were obtained from the herbarium of Nnamdi Azikiwe University, Awka. Chemicals used were as detailed by [11-13].

2.2 Sample Preparation

Except for the isomerised hop extract prepared by Ritchies, each plant sample was milled and vacuum dried at 50 °C. Two kilograms (2 kg) of each plant material thus prepared was stored in a dessicator for the rest of the experiment. Three hundred grams (300 g) each of the resulting powders were then used to obtain the extracts by steeping procedure.

2.3 Ethanol Extraction

The ethanol extract was prepared by steeping 300 g of the dry powdered plant material in 1.5 litres of ethanol at room temperature in a tight fitting round bottom flask for forty eight hours. The mixture was filtered first through a Whatman filter paper (No. 42) and then through a sintered glass funnel. The filtrate was concentrated using a rotary evaporator with water bath set at 60 °C for 2 hours to obtain each extract. The extract was stored in amber colored reagent polypropylene bottle in a deep freezer (Thermofrost, Mod.TR150S) at -5 °C for subsequent use.

2.4 Brewing of Beer

The processes involved in beer brewing namely malting, mashing and fermentation were employed.

2.4.1 Malting

400 g sorghum grain (CSR01) were sorted to remove stones, broken grains, non-uniform sized grains and other foreign materials. 300 g of the sorted grains was washed in a clean bucket and soaked in 900 cm³ of deionized water containing 0.1% formaldehyde to inhibit microbial growth. Steeping was done for 24 hours with 2 hours' air rest between 11th to 13th hours of steeping (i.e. 12 hours water steep, 2 hours air rest and 10 hours water steep in a fresh 900 cm³ of deionized water containing 0.1% formaldehyde). The steeped grains were allowed to germinate in dark chamber for 5 days, with intermittent turning and spraying of 30 cm³ of deionized water 12 hourly to avoid matting and drying up. Germination was stopped by drying the germinated grains in an oven at a temperature of 55 °C for 24 hours (kilning). The kilned grains were de-rooted or deculmed by rubbing in an undulated surface to separate the rootlets. The 'malt' was then weighed and the percentage malting loss-determined as follows:

$$\text{Malting Loss} = \frac{W_{bm} - W_{am}}{W_{bm}} \times \frac{100}{1}$$

where, W_{bm} = Weight of grain before malting; W_{am} = Weight of grain after malting for a given number of germination days.

2.4.2 Mashing

A 200 g of malted grains was milled to coarse particles using a laboratory milling machine (Gibbons, Model 8). The milled malt was mixed with water (800 mL) at 40 °C in a regulated water bath and allowed to stand for 30 minutes with intermittent stirring. The temperature of the mash was increased to 55 °C and the time taken for the mash to reach 55 °C was noted. The mash was allowed to rest at 55 °C for 30 minutes, with stirring every 10 minutes. The temperature of the mash was further raised to 65 °C and the time it took was recorded. The mash was again allowed to rest at 65 °C for 30 minutes, with stirring every 10 minutes. The temperature was then raised to 72 °C, noting the time taken to reach this temperature and allowed to stand for 10 minutes. The temperature was reduced to 60 °C and the time taken for the temperature of the mash to come down to 60 °C was recorded. At this temperature, 5 cm³ of exogenous enzyme (α -amylase-fungamyl) was added and allowed to rest at this temperature for 30 minutes. The temperature was then increased to 75 °C noting the time it took to reach 75 °C and heated for 10 minutes to denature the enzyme. The mash was filtered into 500 cm³ conical flask using Whatman No. 1 filter paper. The volume of the wort recovered was measured. The wort was boiled for 20 minutes.

2.4.3 Fermentation

The wort was divided into 6 portions and cooled to 8 – 10 °C. A 5 g of brewer's yeast, a bottom-fermenting type (*Saccharomyces uvarum*) was inoculated into 100 mL of the 6 wort portions in 250 cm³ fermenting flasks for primary fermentation for 5 days at 8 – 10 °C observing for yeast flocculation. The fermented 'green beer' was carefully decanted into another set of 250 cm³ fermenting flasks. 0.1 mL each of isomerized hop extract, extracts of hop leaf, *G. kola*, *A. indica*, *V. amygdalina* and *G. latifolium* was added to the 6 green beer portions in the fermenting flasks labeled A, B, C, D, E and F respectively. The flasks and contents were kept in a refrigerator at 8 – 10 °C for secondary fermentation /maturation for twenty-one days. Final beer filtration was carried out and the 'matured' beer samples transferred to sample bottles for analysis. The sample bottles that contained the respective beers were labeled accordingly.

2.5 Physicochemical Properties of Beer

2.5.1 Alcohol Content

Distillation method as described by Ceiwryn [14] was employed. A 50 mL sample was measured into 150 mL volumetric flask at 20 °C and washed into a distillation flask using 100 mL of water. The solution was neutralized with 2 mL of 1M NaOH solution. The solution was distilled and the distillate collected. The distillate was cooled to 20 °C and the specific gravity calculated. The alcohol strength was subsequently determined by reference to an Alcoholometric table.

Calculation:

$$\text{Specific gravity} = \frac{X_2 - X_1}{X_3 - X_1}$$

Where,

X_1 = Weight of empty specific gravity bottle

X_2 = Weight of specific gravity bottle + sample

X_3 = Weight of specific gravity bottle + water

2.5.2 Total Acidity

A 25 mL sample was boiled under reflux for 20 minutes to expel CO₂. The condenser was washed down with deionized water to make up to the original volume of the sample (25 mL). The resulting solution was titrated with 0.1M NaOH solution using bromothymol blue as indicator.

$$\text{Percentage total acidity} = \frac{\text{Titre value} \times \text{Factor equivalent}}{\text{Volume of sample}}$$

Factor equivalent of acetic acid = 0.006.

2.5.3 pH

pH was measured by Electrometric method using laboratory pH meter as described by Food Compliance Laboratory Unit of National Agency for Food and Drug Administration and Control [15]. The electrodes were rinsed with distilled water and blot dried. The pH electrode was then rinsed in a small beaker with a portion of the sample. Sufficient amount of the sample was poured into a small beaker to allow the tips of the electrodes to be immersed to a depth of about 2 cm. The electrode was at least 1 cm away from the sides and bottom of the beaker. The temperature adjustment dial was adjusted accordingly. The pH meter was turned on and the pH of the sample recorded.

2.5.4 Turbidity

AOAC method 970.14 [15] was selected. A 2 mL sample was placed in a clean, dry turbidity vial and capped securely. Excess liquid or fingerprint was wiped off with a soft cloth. The vial and content was placed into the AQ4500 sample chamber. The measure key was then pressed and the result displayed on the instrument with NTU.

NTU= Nephelometric Turbidity Unit.

2.5.5 Total Solids

The method employed was as detailed by Food compliance laboratory unit of National Agency for Food and Drug Administration and Control [16]. The Satorious moisture analyzer was switched on from the mains till off is displayed on the dash board. The (1/Q) ON/OFF key was pressed to display the satorious logo followed by the 0.000 g and the heating programmer. The instrument was allowed to warm up and stabilize for at least 30 minutes in order to reach 105 °C. The sample chamber was

opened to position a new sample pan draft shield on the pan support. The sample pan was tarred (zeroed) by selecting the tare function as needed to get 0.000 g on the dash board and the ENTER key pressed. The prepared sample was weighed and spread on the pan draft shield uniformly. The sample chamber was closed and the drying programmer started when the ENTER key was pressed. The drying programmer shuts off automatically once no further moisture or weight loss is detected.

The percentage weight loss due to the amount of moisture loss is displayed automatically and the percentage moisture loss is recorded.

$$\text{Total Solids} = 100 - \% \text{ moisture loss}$$

2.5.6 Micro Metals (Arsenic, Cadmium and Copper)

Before the analysis, all beer samples were degassed using an ultrasonic bath for 30 minutes. A 10 mL aliquot of the degassed sample was mixed with 2 mL of concentrated nitric acid and 2 mL of hydrogen peroxide in a digestion tube. The mixture was heated for 1 hour at 100 °C until complete clarification and allowed to cool, and filtered and diluted to 25 mL with deionized water. Analytical blanks were prepared in a similar manner, but omitting the test sample. The solutions were subsequently analyzed for arsenic, cadmium and copper using atomic absorption spectrophotometer.

2.5.7 Bitterness Level

Bitterness was determined according to ASBC Beer 23A [17]. Ten millilitre of decarbonated and foam freed beer sample was measured into a 50 mL centrifuge tube. To this was added 1 mL 3M hydrochloric acid and 20 mL iso-octane. The tube was stoppered and agitated for 15 minutes. The tube was further centrifuged for 3 minutes at 3000 rpm. An iso-octane blank was prepared into a 1 cm quartz curvet. A clear iso-octane phase in the centrifuge tube was pipetted into another curvet and stoppered.

The spectrophotometer's λ was set at 275 nanometer and zeroed with the blank before the absorbance of the sample was read. The reading was multiplied by 50 and the result expressed as:

$$\text{Absorbance at 275 nanometer} \times 50 = \text{Bitterness in IBU}$$

where, IBU = International Bitterness Unit.

2.5.8 Statistical Analysis

Tables were generated using the Schimadzu GC – MS solution software and MS library. From triplicate experiments in phytochemical screening and metal content analysis of extracts, and the investigation of physicochemical properties of the brewed beer samples, the mean, standard deviation and range in all the studies were evaluated. The mean values were used to construct bar charts for easy interpretation of results. Simple statistics (ranking) was employed to determine the significant difference between the controls (hops) and the Nigerian vegetables. In ranking, we determined the existence of the significant difference among isomerized hop (control), *G. kola*, *A. indica*, *V. amygdalina* and *G. Latifolium* on one hand and hop leaf (control), *G. kola*, *A. indica*, *V. amygdalina* and *G. latifolium* on the other hand. We employed test of significant difference using Analysis of Variance (ANOVA). The software used for the analysis was SPSS (Special Package for Social Sciences) Version 20.

2.5.9 Ranking

In the test of significant difference, One Way Analysis of Variance (ANOVA) is the most suitable tool as it has the capacity to show the existence of difference at 5% level of significance [18]. In ANOVA, two hypotheses, H_0 and H_1 are stated and tested for:

H_0 : there is no significant difference among samples of interest.

H_1 : there is significant difference among samples of interest.

The result of the p- value (significance value) is used to accept or reject either of the hypotheses.

3. Results and Discussion

3.1 Malting

The results of malting show a decrease in weight of the grains from 300 g to 281.19 g, Table 1, after five days of germination representing a malting loss of 6.27%.

Table 1 Malting Loss for CSR01 Sorghum Variety

Weight of Grain after Sorting (g)	Weight of Grain after 5 days of Germination (g)	Malting Loss (%)
300	281.19	6.27±0.524

This result is in agreement with that obtained by Archibong et al. [19] in their malting properties of SSV 200504 sorghum variety. Those authors had reported a malting loss of 5.60% after 5 days of germination (Table 2).

Table 2 Malting loss for SSV 200504 Sorghum Variety [19]

Days of Germination	Malting Loss (%)
1	3.80
2	4.20
3	4.62
4	4.92
5	5.60
6	5.88

3.2 Mashing Programme

Table 3 shows that the time taken by the mash to get from 40 °C to 55 °C; 55 °C to 65 °C; 65 °C to 72 °C; 72 °C to 60 °C and 60 °C to 75 °C is 12 minutes, 10 minutes, 15 minutes, 25 minutes and 10 minutes respectively.

Table 3 Temperature regime, time taken to attain the temperature and rest time at attained temperature

Temp. Regime (°C)	Time taken (mins)	Rest time (mins)
25-40	-	30
40-55	12	30
55-65	10	30
65-72	15	10
72-60	25	30
60-75	10	10

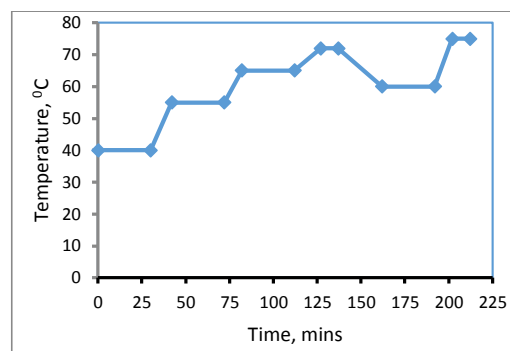


Fig. 1 Mashing regime for wort production

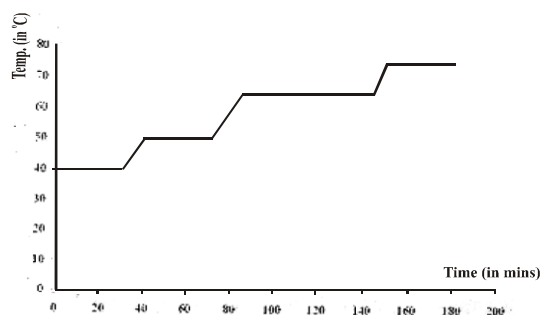


Fig. 2 Mashing Regime for SSV 200504 Sorghum variety [20]

Fig. 1 shows that mashing of the milled malt starts at 40 °C and rested for 30 minutes at this temperature. It took 12 minutes (30-42) before it attained a temperature regime of 55 °C and rested for 30 minutes (42-72). It took the mash another 10 minutes (72-82) to attain the temperature of 65 °C where it was allowed to rest for 30 minutes (82-112) before it was raised to 72 °C after an interval of 15 minutes (112-127). The mash rested at 72 °C for 10 minutes (127-137) before the temperature was reduced to 60 °C. The time taken to attain this temperature is 25 minutes (137-162) and allowed to rest for 30 minutes (162-192). It took another 10 minutes (192-202) to attain a higher temperature of 75 °C where it was allowed to

rest for 10 minutes [202–212]. The results in Fig. 1 are consistent with that of Archibong and Onuorah [20], Fig. 2 indicating that there is no significant difference in the mashing regimes.

3.3 Physical and Chemical Properties of the Beer Samples

3.3.1 Alcohol Content

Table 4 shows that percentage alcohol was virtually in the same range in all the samples especially samples A and D with alcohol content of 3.75 %v/v. All the other samples contained equal alcohol content of 3.43 %v/v.

This result is in agreement with the report of Hough et al. [3] in their investigation of alcohol content in British beers. The authors had reported that the majority of beers tested contained between 2.5 %v/v and 5 %v/v alcohol. However, none of the alcohol content of all the beer samples is up to Nigeria's National Agency for Food and Drug Administration and Control (NAFDAC) maximum allowed alcohol content of 5 %v/v. The alcohol content of beer is usually regarded as the measure of its strength and therefore, the more the alcohol content, the stronger the beer and vice versa. The comparability of the results indicates that fermentation of the wort was done under the same condition and that hops do not affect the alcohol content of beers.

Table 4 Physicochemical Properties of the Brewed Beer Samples

Parameter	Beer Sample					
	A	B	C	D	E	F
Alcohol (%v/v)	3.75	3.43	3.43	3.75	3.43	3.43
Total Acidity (%)	0.228	0.144	0.132	0.324	0.288	0.288
pH at 24 °C	5.57	5.68	5.57	5.47	5.50	5.49
Turbidity (NTU)	50	100	110	125	5	6
Total Solids (%)	6.34	3.56	3.86	8.16	4.72	5.85
Arsenic (ppm)	1.50	1.44	1.77	1.52	1.62	1.60
Cadmium (ppm)	0.00	0.81	0.97	0.96	0.00	0.68
Copper (ppm)	2.70	1.99	2.39	0.10	1.32	1.46
Bitterness level (IBU)	39.62	30.91	25.38	33.87	29.12	27.56

3.3.2 Total Acidity

The total acidity in all the beer samples varied between 0.324% and 0.132% with the highest percentage of total acidity in sample D being 0.324% and lowest value of 0.132% in sample C. Table 4 shows that percentage total acidity in samples A and B are respectively 0.228% and 0.144%, whereas that in samples E and F contain equal content of total acidity of 0.288% each. It is therefore evident that the percentage total acidity of all the samples are comparably within the same range. Interestingly, all the beer samples exceeded the 0.1% minimum allowed total acidity of NAFDAC's recommendation in lager beers in Nigeria [16].

3.3.3 pH

Table 4 shows that the pH of the beer samples are comparably the same. Sample B has the highest pH of 5.68 (less acidic). Sample A and C have the same pH value of 5.57. Sample D has the lowest pH value of 5.47 (more acidic) while samples E and F have pH values of 5.50 and 5.49 respectively. This is an indication that all the samples could substitute one another in beer brewing. Interestingly, the pH values are within the NAFDAC standard for lager beers. Worts with pH (5.0 – 5.5) have better protein precipitation and break formation [21].

3.3.4 Turbidity

The turbidity of all the samples as presented in Table 4 ranged between 125 and 5 NTU. Sample E was virtually the clearest and D, the most turbid (cloudy). Samples B, C, and D were 100, 110, and 125 respectively, all in Nephelometric Turbidity Unit (NTU) and were especially high compared with the turbidity in samples E (5 NTU) and F (6 NTU). The turbidity in sample A was 50 NTU. These results are not in agreement with the turbidity standards (0.15 NTU) for drinking water in the United State [22]. These results show that the turbidity in samples B, C and D are comparable to one another; samples E and F are also comparable to each other while sample A is not comparable to any of the samples. These discrepancies that exist in turbidity values of the beer samples could easily be explained by the fact that length of time each beer sample was exposed to the atmosphere during hopping was not constant. During each period, fugitive harmful organisms such as bacteria, viruses, protozoa, moulds, and wild yeasts could infect the beer. The more the beer is exposed to the atmosphere, the more the loads of these organisms and of course the more the beer develops a biological haze and goes turbid. These results could

explain the reason why excess consumers of beer often complain about gastrointestinal diseases because in drinking water, the higher the turbidity level, the higher the risk that people may develop gastrointestinal diseases [23].

3.3.5 Total Solids

From Table 4 the percentage total solids ranged between 3.86 and 8.16 with sample D having the highest percentage of 8.16 and the lowest, sample B, with a value of 3.86%. The percentage total solids of the beer in samples B, C, and E are within the permissible maximum limits of total solids in beer. The National Agency for Food and Drug Administration and Control (NAFDAC)'s permissible maximum unit of total solids in beer is 5%. The percentage total solids in samples A and D are above the permissible limit while that in F is slightly above the limit. These results show that the total solids in sample D were more than twice those in samples B and C. This observation reveals that hops contribute to percentage total solids in beer. Total solids in A, E, and F are virtually within the same range. However, the results are in agreement with the report of O'Rourke [24] on water content of beer. O'Rourke has reported that beers contain more than 90% water.

3.3.6 Arsenic, Cadmium and Copper

The results from Table 4 show that the concentrations of these elements in the finished beer samples differ among themselves because metals in beer are derived from various raw materials, equipment and brewing processes [25].

3.3.6.1 Arsenic

From Table 4, arsenic concentration in the samples ranged between 1.44 – 1.77 mg/L, with sample C having the highest concentration of 1.77 mg/L, and sample B, the lowest concentration of 1.44 mg/L. The FAO/WHO maximum permissible limit of arsenic in drinking water is 10 µg/L [26]. The arsenic content of the beer samples was above the maximum permissible limit of arsenic in drinking water. In Britain, the level of arsenic in lagers may not exceed 0.2 mg/kg [3]. Again, the concentration of arsenic in the beer samples investigated is much more above this level. The explanation for this may be the region of growth of the raw materials used in the production.

3.3.6.2 Cadmium

From the results in Table 4, cadmium concentration in the beer samples ranged between 0.97 ppm and not detected with sample C having the highest concentration of 0.97 mg/L and not detected in samples A and E. The concentrations of cadmium in samples B, D and F are 0.81 ppm, 0.97 ppm and 0.68 ppm respectively. These results differ significantly with the result of Ubuoh [27] except in samples A and E. World Health Organization [21] reported a cadmium content varying from 12.90–14.30 µg/L in Brazilian beers. Also, the Standard Organization of Nigeria [28] gave the limit for Cd content in drinking water as 1µg/kg bw/day. All the beer samples examined had Cd concentrations above that in Brazilian beers and the permissible limit in drinking water with the exception of beer samples A and E where Cd was not detected.

3.3.6.3 Copper

Copper content of the beer samples as shown in Table 4 varied between 2.70 ppm and 0.10 ppm, with sample A having the highest concentration of 2.70 ppm, and the lowest being sample D with 0.10 ppm. The permissible limit for copper in drinking water in Nigeria is 1.0 ppm [28], [29] and in Britain, the Food Standard Committee has recommended limits of 7.0 ppm and 5.0 ppm for copper and zinc respectively in wines and beers [3]. The Copper content of the beer samples analyzed was above the permissible limit for drinking water in Nigeria except in sample D but below the limit in British beers.

3.3.7 Bitterness Level

The bitterness level in all the samples ranged between 25.38 IBU and 39.62 IBU with sample A having the highest bitterness level of 39.62 IBU and sample C, the lowest bitterness level of 25.38 IBU. Samples B, D, E and F have bitterness levels of 30.91 IBU, 33.87 IBU, 29.12 IBU and 27.56 IBU respectively. Table 4 shows that bitterness level in all the beer products are virtually in the same range and especially high in sample A. These results are consistent with the report of Ashurst [30] that non-polar fat solvents are suitable for the bittering constituents of hops and that bitterness level in beers depends on the age and method of storage of hops used in brewing.

3.3.8 Ranking

3.3.8.1 Isomerized Hop

The p-value of the test as shown in Table 5 is 0.705 which is greater than 0.05. We then have enough evidence to accept the null hypothesis and conclude that there is no significant difference among the samples studied.

Table 5 ANOVA for comparison of physicochemical properties of beers brewed with isomerized hop and the tropical plants

	Sum of squares	Df	Mean square	F	Sig.
Between Groups	1497.217	4	374.304	0.543	0.705
Within Groups	27565.760	40	689.144		
Total	29062.978	44			

The output of the post Hoc Test shows that *G. kola* has the highest significance value of 0.696 which implies that the plant is the closest among all the samples to isomerized hop. Other plants (*A. indica*, *V. amygdalina* and *G. latifolium*) have significance values less than 0.696 but greater than 0.05. This implies that all the plants are insignificantly different from isomerized hop.

3.3.8.2 Hop Leaf

From Table 6, it is seen that the p-value of the test is 0.734 which is greater than 0.05. We therefore have enough evidence to accept the null hypothesis and conclude that there is no significant difference among the plants considered.

Table 6 ANOVA for comparison of physicochemical properties of beers brewed with hop leaf and the tropical plants

	Sum of squares	Df	Mean square	F	Sig.
Between Groups	1632.103	4	408.026	0.490	0.743
Within Groups	33324.703	40	833.118		
Total	34956.806	44			

It is seen from post Hoc Test that in the comparison of hop leaf with the tropical plants, *G. kola* has the highest significance value of 0.964 which implies that *G. kola* is the closest among the plants to hop leaf (control). Other plants (*A. indica*, *V. amygdalina* and *G. latifolium*) have significant values less than 0.734 which are higher than 0.05. This means that the plants are not significantly different from hop leaf.

4. Conclusion

The physical and chemical parameters [total solids, alcohol content, pH, turbidity, total acidity, inorganic micro metals (arsenic, cadmium and copper) and bitterness level investigated in the beers brewed with extracts from tropical plants showed no significant difference from those of the controls.

It was established from ranking that the order of closeness of the vegetables investigated to isomerized hop extract was *G. kola* (0.969) > *G. latifolium* (0.609) > *V. amygdalina* (0.601) > *A. indica* (0.536) while that to hop leaf extract was *G. kola* (0.964) > *A. indica* (0.800) > *G. latifolium* (0.440) > *V. amygdalina* (0.433).

This study has shown that the extracts from tested Nigerian plants could be used as suitable substitutes for hops in beer brewing without alteration of the physicochemical properties of beer. Extract of *G. kola* had the greatest potential as substitute for imported hops.

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References

- [1] T. Goldamer, *Brewers Handbook*, Apex Publishers, USA, 2008.
- [2] L. Badmus, Beer and the Nation's Economy-The Nigerian Telegraph, 2013. <http://telegraphing.com/2013/11/beer-nations-economy>. (Accessed on: 25-10-2014).
- [3] J.S. Hough, D.E. Briggs, R. Stevens, T.W. Young, *Malting and brewing science*, 2nd Ed., Chapman and Hall, London, England, 1982, pp. 389-452.
- [4] R.A. Shellie, S.D.H. Poynter, J. Li, J.L. Gathercole, S.P. Whittlock, et al, Varietal characterization of hop (*Humulus lupulus* L.) by GC-MS analysis of hop cone extracts, *J. Sep. Sci.* 32 (2009) 3720-3725.
- [5] D.R.J. Laws, Hops, *Brewer's Guardian* 110 (5), Institute of Brewing, London, 1981, pp. 71-74.
- [6] N. Okafor, G. Anichie, G. West, African hop substitute for sorghum lager, *Brewing Distil. Int.* 1(3) (1983) 20-21.
- [7] P.E. Ajebesone, J.O. Aina, Potential African substitutes for hops in tropical beer brewing, *J. Food Technol. Afr.* 9(1) (2006) 13-16.
- [8] M.M. Iwu, O.A. Igboke, C.O. Okunji, M.S. Tempesta, Antidiabetic and aldose reductase activities of Biflavanones of *Garcinia kola*, *J. Pharm. Pharmacol.* 42 (2012) 290-292.
- [9] G.C. Akudor, M.S. Idris-Usman, C.C. Mba, U.A. Meqwas, J.L. Akpan, et al, Studies on anti-ulcer, analgesic and antipyretic properties of the ethanolic leaf extract of *Gongronena latifolium* in rodents, *Afr. J. Biotechnol.* 9(15) (2010) 2316-2321.
- [10] B. Joshi, G.P. Sah, B.B. Basnet, M.R. Bhatt, D. Sharma, et al, Phytochemical extraction of antimicrobial properties of different medicinal plants, *Ocimum sanctum* (tulsi), *Azadirachta indica* (neem), *Eugenia caryophyllata* (dove) and *Achyranthes bidentata* (datiwan), *J. Microbio. Antimicrobials* 3(1) (2010) 1-7.
- [11] AOAC, Official methods of food analysis, 19th Edition, Association of Official Analytical Chemists, Washington, D.C., 1980.
- [12] ASBC, *Methods of Analysis*, 7th Ed., American Society of Brewing Chemists, St. Paul, Minnesota, 1976.
- [13] IOB, *Recommended methods of analysis*, The Institute of Brewing, London, 1977.
- [14] S.J. Ceirwyn, *Analytical Chemistry of Foods*, An ASPEN Publication, Gaithersbury, Maryland, 1999, pp. 162-164.
- [15] AOAC, AOAC Method 970.14, Haze (Total) of Beer after Chilling, Association of Official Analytical Chemists, Washington D.C., 2000.
- [16] NAFDAC, National Agency for Food and Drug Administration and Control, Regulations in larger beers produced in Nigeria, National agency for food and drug administration and control, Abuja, Nigeria, 2012.
- [17] ASBC, American Society of Brewing Chemists Beer-23A, American Society of Brewing Chemists, Minnesota, 1999.
- [18] S.C. Gupta, *Fundamentals of statistics*, Smith revised and enlarged edition. Himalaya publishing house, Delhi, Mumbai, 2011, pp. 1-23, 37.
- [19] E.J. Archibong, F.J.C. Odibo, N.S. Awah, Effects of germination days on the malting properties of three (3) new sorghum varieties (ICSVIII, SSV200504 and SSV200503), *World J. Biotechnol.* 40(1) 2009 1578-1583.
- [20] E.J. Archibong, S.C. Onuorah, Effects of mashing regime on wort characteristics of three improved sorghum varieties (ICSV III, SSV200504 and SSV200503), *J. Appl. Sci.* 13(1) (2010) 9311-9319.
- [21] T. Kraus-Weyerman, pH in the Brewery, A much underestimated Brewing Variable, Weyermann Malting Company, Germany, 1998, pp. 1-30.
- [22] EPA, Drinking water contaminants, United State Environmental Protection Agency, Washington, D.C., 2009.
- [23] A.G. Mann, C.C. Tam, C.D. Higgins, L.C. Lodrigues, The association between drinking water turbidity and gastrointestinal illness: a systematic review, *BMC Public Health* 7(256) (2007) 1-7.
- [24] T. O'Rourke, The treatment and use of water in brewing, *Brewers' Guardian*, 127 (12), Institute of Brewing, London, 1998.
- [25] WHO, Safety evaluation of certain food additives and contaminants: Cadmium, WHO Food additives series 46: Joint FAO/WHO Expert Committee on Food Additives, World Health Organization, Geneva, 2011.
- [26] FAO/WHO, Evaluation of certain contaminants in food. Seventy second report of the joint FAO/WHO expert committee on food additives: World Health Organization (WHO) technical report series, No. 959, Geneva, 2011.
- [27] E.A. Ubuoh, Analysis of metal concentrations in selected canned beers consumed in Owerri Urban, Imo State, Nigeria, *Int. J. Chem. Mater. Sci.* 1(15) (2013) 090-095.
- [28] SON, Standard Organization of Nigeria, Safe drinking water regulation, Standard Organization of Nigeria: Abuja, Nigeria, 2013.
- [29] WHO, World Health Organization evaluation of certain food additives and contaminant, technical report series no. 837, World Health Organization, Geneva, 2007.
- [30] P.R. Ashurst, Hops and their use in brewing in: W.P.K. Findlay (edited), *Modern brewing technology*, The Macmillan Press, Cleveland, Ohio, 1971, p. 144.