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Full Length Research Paper

Utilization of bitter vegetable leaves (*Gongronema latifolium*, *Vernonia amygdalina)* and *Garcinia kola* extracts as substitutes for hops in sorghum beer production

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Sorghum beer was brewed using extracts of 'utasi' leaf *(Gongronema latifolium)*, bitter leaf (*Vernonia amygdalina)* and bitter kola (*Garcinia kola)* to impact bitter taste and flavour as substitutes for hops used for beer production. The physicochemical and sensory characteristics of the beer samples were evaluated. The results showed that 'utasi' leaf, bitter leaf and bitter kola flavoured beer samples had alcohol values in the range of (12.50 - 12.55%), pH (3.37 - 4.44), specific gravity (1.0140 - 1.0400), titratable acidity (0.107 - 0.328%), volatile acidity (0.003 - 0.103%), soluble solids (0.12 - 0.97%), moisture content (95.71 - 98.37%), protein (0.17 - 0.85%), ash (0.20 - 0.29%) and fat (1.25 - 2.95%). Sensory evaluation of the beer by trained panelists (connoisseurs) showed that the utasi flavoured beer was preferred to the other beer samples and compared favourably with hopped beer in terms of flavour and taste.

Key words: Beer production, 'utasi' leaf, bitter kola, bitter leaf, hops substitutes.

INTRODUCTION

Hops are the dried female flowers of the plant *Humulus lupulus* and *Homarus americanus,* a perennial in the cannabinaceae family. They are climbing plants which grow every spring from overwintering root stock (Laws, 1983). The hop plant is dioecious, having separate male and female plants (Smith, 1979). The hops provide desirable aromatic components and bitterness (Goldamner, 1990). In earlier times when beer was not made in summer due to rapid spoilage, there had been a stability advantage in hopped beer over un-hopped beer. Hop components have been shown to inhabit a wide variety of gram positive bacteria and some fungi but have no effect on yeast. Hop efficiency is defined as the amount of hops bitter compounds extracted into the wort. Hop is composed of flavour compounds of aromatic essential oil, bitter resin as well as amino acids (Briggs et al., 1981).

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The resin can be separated into hard resin (insoluble in hexane) and soft resin. The soft resins contribute the bitter substances (humulone or α-acids and lupulone or β-acids) (Ajebosome and Aina, 2004). Hops contribute to foam stability and also provide hop flavour, hop character and preservative properties to the beer (Laws, 1983). The use of bittering agents in brewing to produce the characteristic flavour, preservative properties and foam stability to beer has recorded a tremendous advantage from its modest beginning (Sueeri, 1991). Many other plants have been used for bittering or flavouring beer in various parts of the world (Smith, 1979). *Gorgronema latifolium,* commonly called 'utasi' and 'arokeke' in the South Eastern and South Western Nigeria, respectively, is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine (Ugochukwu and Babady, 2002; Ugochukwu et al., 2003). Reports by various authors showed that it contains essential oils, saponins and pregnanes among others (Schneider et al., 1993; Morebise and Fafunso, 1998).

Ugochukwu and Babady (2003), Ugochukwu et al.

(2003) and Ogundipe et al. (2003) reported that aqueous and ethanolic extracts of *G. latifolium* had hypoglycemic, hypolipidemic and antioxidative properties while showed that it has anti-inflammatory proper-ties. Bitter leaf *(Vernonia amygdalina)* is derived from the leaves of a small ever-green shrub found all over Africa belonging to the family Asteraceace. It is well known as a medicinal plant for diabetes and fever (Crellin et al., 1989). Bitter kola *(Garcinia kola)* is cultivated and distri-buted throughout West and Central Africa. Its medicinal uses include anti-parasitic, antimicrobial and purgative purposes (Ross, 2001). The constituents include biflavo-noids, xanthones and benzophenones (Iwu, 1993).

Information on the use of indigenous bitter vegetables has not however received enough attention in brewing industries in Nigeria. The objective of this study therefore is to substitute commercial hops with Nigerian bitter vegetables namely, *G. latifolium*, *V. amygdalina* and *G. kola* in lager beer production and to evaluate their physical, chemical and sensory qualities.

MATERIALS AND METHODS

Procurement of raw materials

Sorghum *(Sorghum bicolor),* bitter leaf (*V. amydalina*) and 'utasi' (*G. latifolium)* were purchased from Sabo market in Ikorodu, Lagos, South West Nigeria. Hop was obtained from Sona Breweries, Sango Ota, Ogun State, South West Nigeria. Bitter kola (*G. kola)* was obtained from Shibodo Farm, Ikorodu, Lagos.

Extraction of bitter components from bitter vegetables and bitter kola

The bitter components from bitter vegetables and bitter kola were extracted using the methods of Oshodi et al. (2004) and Ross (2001). Ten grams of fresh vegetable leaf was washed with tap water, oven-dried (Gallenkamp Co. Ltd. London), at 50°C for 24 h, cooled, dried-milled, sieved, packed in high density polythene bags and stored at room temperature before use. To obtain vegetable extracts, ten grams of fresh leaves was blended with one hundred millilitres water and sieved to obtain the extracts and used immediately. Bitter kola coat was scrapped with a knife, sliced thinly, dried in an oven, milled into powder, sieved and packaged into polythene bags before use. The bitter kola extract was obtained by boiling one hundred grams powdered bitter kola in three hundred millilitres of water for 30 mi, sieved and concentrated using rotary evaporator to one hundred millilitres before use.

Production of beer

Malting

Malted sorghum was prepared by the method described by Withy and Lodge (1985) with slight modification. The steeping time was 48 h instead of 24 h at 10 to 15.6°C and the water was discarded every 8 h. The grains were germinated on clean stainless steel container loosely covered with muslin cloth at 16°C for five days. Kilning was carried out using moderate heat of 50 - 70°C, turning the malt to aerate, avoiding local overheating and achieving uniform controlled heat. Kilning stopped the sprouting and the rootlets were removed. The malted sorghum was crushed using a milling machine to produce grits.

Mashing and fermentation

The method for mashing as described by Sueeri (1991) was adapted and used. About 310 g of sorghum malt was mixed with 14.41 L warm water at 68°C. The mixture was heated and passed through sieve mesh screen at 65°C. To the mash in the sieve was added warm water (50°C) to extract the enzymes from the grit. The wort was divided into six portions of 2 L each. The different vegetables and bitter kola extracts and powder were added separately to each of the first five portions as hops substitutes and boiled for 2 h. Another 2 L of wort sample was flavoured with hop extract (control) and boiled for 2 h. The different wort samples after boiling were made up separately to 80% of their initial volume with water, cooled to 12°C, pitched with ten grams yeast *(Saccharomyces cerevisiae)* and allowed to ferment at 12°C for 12 days. The beer samples were finally aged for six months.

Analyses of beer samples

Each beer sample was analyzed for pH, specific gravity, alcohol content, titratable acidity, soluble solids, moisture content, ash, fat and protein. The pH was determined with a digital pH meter at ambient temperature (30 ± 2°C). The specific gravity was determined by the use of specific gravity bottle at a temperature of 28.2°C. Alcoholic content was determined with alcohol hydrometer as recommended by the Institute of Brewing (IOB, 1984). Titratable acidity (% lactic acid) was measured by titrating 10 ml sample with 0.1N NaOH to phenolphthalein end point (James, 1999). Soluble solids, ash, fat and protein were analyzed according to AOAC (1980) methods. All the chemicals used were of analytical grade. All determinations were carried out in triplicates.

Sensory evaluation

Sensory evaluation was carried out using 30-member panel consisting of Staff and Students of the Department of Food Technology, Lagos State Polytechnic, Ikorodu, Lagos. The panelists ranged between 18 and 35 years of age. A 9 – point hedonic rating scale was used. All data were subjected to analysis of variance (ANOVA) and mean separated with Duncan multiple range test using SAS programme (SAS, 1985).

RESULTS AND DISCUSSION

The results of the physical and nutritional properties of the beer samples flavoured with hop and various vegetable extracts are presented in Table 1. The data indicated that the alcohol contents of all samples were not significantly ($P < 0.01$) different. The alcohol contents ranged from 12.50 to 12.55% v/v while the pH and titratable acidity ranged from 3.37 to 4.44 and 0.107 to 0.328%, respectively. The specific gravity and the soluble solids ranged from 1.0140 to 1.0400 and 0.12 to 0.97, respectively. The protein contents ranged from 0.17 to 0.85% while ash and fat ranged from 0.20 to 0.29% and 1.25 to 2.95%, respectively. The proximate values of the beer samples compared with those previously reported

Table 1. Physical and nutritional properties of the beer samples flavoured with various vegetable extracts.

Parameter	Beer sample					
	HOP	A	B	C	D	E
Alcohol content (%)	12.55	12.52	12.50	12.53	12.51	12.50
pH (at 30°C)	4.44	3.41	3.42	4.06	3.37	3.42
Titratable acidity (%)	0.107	0.140	0.118	0.111	0.328	0.114
Volatile acidity (%)	0.103	0.039	0.054	0.034	0.003	0.032
Specific gravity	1.0161	1.0180	1.0230	1.0140	1.0206	1.0400
Soluble solids (%)	0.97	0.12	0.14	0.17	0.12	0.18
Protein (%)	0.68 ± 0.3	0.34 ± 0.4	0.68 ± 0.8	0.85 ± 0.2	0.17 ± 0.6	0.34 ± 0.1
Moisture content (%)	95.71 ± 2.1	98.37 ± 0.2	95.95 ± 1.6	97.73 ± 1.9	97.22 ± 0.1	97.14 ± 1.4
Ash (%)	0.26 ± 0.4	0.20 ± 0.1	0.20 ± 0.1	0.29 ± 0.5	0.23 ± 0.1	0.24 ± 0.7
Fat (%)	1.25 ± 0.2	1.80 ± 0.2	2.78 ± 0.0	2.95 ± 0.3	1.95 ± 0.1	2.20 ± 0.7

A, Sample flavoured with bitter kola powder; B, sample flavoured with bitter kola extract; C, sample flavoured with 'utasi' extract; D, sample flavoured with bitter leaf extract; E, sample flavoured with bitter leaf powder.

by several authors (Ajebosome and Aina, 2004; Mbah et al., 1981; Smith, 1979; Eka, 1984).

Okafor and Anichie (1983) reported an average of 10.8% alcohol, 1.081 specific gravity and 1.39 soluble solids for beer produced from 8 - 10 days malted sorghum grains. Perisse et al. (1995) investigated the composition of traditionally brewed sorghum beer fermented for 72 h in Togo and reported an average of 3.03% alcohol, 0.3% protein and 0.2% ash. In their work on the traditional preparation of sorghum beer (amgba) in Cameroon, Chevassus-Agnes et al. (1976) reported an average of 2.7% alcohol, 90.7% moisture content, 0.7% protein, 0.3 fat and 4.06 ash. Novellie (1977) investigated the nutritional contents of Bantu sorghum beer in South Africa and reported an average of 3.0% alcohol, 4.0% soluble solids and 0.6% protein.

In this study, the alcohol content of 'utasi' flavoured sorghum beer (12.53%) was the closest to the value for hopped beer (12.55%) indicating that fermentation of sugar was more in these beer samples than other samples. The alcohol contents were slightly higher than the values reported by Okafor and Anichie (1983). The major change that occurs during fermentation of beer is the conversion of fermentable sugars (monosaccharide and disaccharide) to alcohol and carbon dioxide. The pH values of the beer fell due to formation of carbon dioxide and organic acid, mostly lactic acid. After fermentation, the pH of the hopped beer (4.44) was higher than those of bitter kola powder beer (3.41), bitter kola extract beer (3.42), 'utasi' beer (4.06), bitter leaf extract beer (3.37) and bitter leaf powder beer (3.42). This showed that all the samples were acidic. The pH values in previous studies have been reported in the range of 3.3 to 3.5 in sorghum beer samples (Eka, 1984; Smith, 1979).

This situation conferred more stability to the beer samples. Higher beer Ph influences flavour, improves fining action but makes beer susceptible to bacterial contamination (Mbah et al., 1981).

The specific gravity and soluble solids of all the samples were not significantly ($P < 0.01$) different. The specific gravity of the beer samples ranged between 1.0140 in 'utasi' extract flavoured beer and 1.0400 in bitter leaf powder flavoured beer. The breakdown of the wort components was monitored by taking the specific gravity of the wort as fermentation progressed. The soluble solids of hopped beer (0.97) was higher than bitter leaf powder flavoured beer (0.18), 'utasi' extract flavoured beer (0.17), bitter kola extract beer (0.14), bitter kola powder beer (0.12) and bitter leaf extract beer (0.012) indicating that not all of the fermentable sugar was fermented in all the beer samples. These results are in conformity with the findings of Ajebosome and Aina (2004), Smith (1979), Okafor and Anichie (1983). Fermentation is considered to be complete when the desired degree of sugar conversion called attenuation has taken place (Withy and Lodge, 1985).

The extracts added to the sorghum beer increased the nutritive values of the beer. The protein content ranged between 0.85% in 'utasi' flavoured beer and 0.17% in bitter leaf extract flavoured beer while the fat content ranged between 2.95% in 'utasi' beer and 1.25% the hopped beer. The values of protein, ash and fat of the beer samples were similar to the values reported by Perisse et al. (1995) and Chevassus-Agnes et al. (1976) in traditionally brewed beer from sorghum in Togo and Cameroon, respectively. Some of the beer samples brewed with vegetable extracts had poor foam heads. An important property of beer is its head or foam retention which should cling to the side of the glass and not disappear too rapidly when poured (Sueeri, 1991; Ajebosome and Aina, 2004).

The higher molecular weight constituents particularly

Table 2. Measurement of foam stability and clarification of beer samples

Sample	Hops	A	B	C	D	E
Foam stability period (sec)	148	122	37	8	60	50
Period of clarification (sec)	72	18	22	100	17	19

A, Sample flavoured with bitter kola powder; B, sample flavoured with bitter kola extract; C, sample flavoured with 'utasi' extract; D, sample flavoured with bitter leaf extract; E, sample flavoured with bitter leaf powder.

Table 3. Sensory characteristics of beer samples.

Sample	Colour	Taste	Flavour	Mouth feel	Overall acceptability
A	$7.0 \pm 0.39^{b*}$	6.9 ± 0.45	6.6 ± 3.38	7.3 ± 0.2	7.0 ± 0.09^{b}
B	7.0 ± 0.88^{b}	7.3 ± 0.55^{ab}	7.4 ± 0.69^{a}	7.4 ± 0.01	7.1 ± 0.83^{b}
C	8.3 ± 0.74^{ab}	8.3 ± 0.63^{a}	8.6 ± 0.44^{ab}	8.0 ± 1.93	7.6 ± 1.21^{ab}
D	7.2 ± 0.8^{a}	7.6 ± 0.61^{a}	7.7 ± 0.98^{a}	7.9 ± 0.7	7.2 ± 0.70
E	7.3 ± 0.09^{b}	7.5 ± 0.83^{b}	7.6 ± 1.21^{ab}	7.7 ± 0.76^{a}	7.3 ± 0.01
Control	8.4 ± 0.65	8.1 ± 1.03^{a}	8.5 ± 0.77^{ab}	8.3 ± 0.11	7.5 ± 0.76^{a}

A, Sample flavoured with bitter kola powder; B, sample flavoured with bitter kola extract; C, sample flavoured with 'utasi' extract; D, sample flavoured with bitter leaf extract; E, sample flavoured with bitter leaf powder.
*Means with the same superscripts in a column are not significantly different ($P < 0.05$).

protein, malt gums and hop resins together with carbonation are responsible for the degree of foam formation (Briggs et al., 1981). Because of its colloidal nature, hop contributes to the foam head retention and increases the body of the beer (Okafor and Anichie, 1983). This explains why hopped beer had the highest foam stability period (148 s), (Table 2) and utasi flavoured beer had the least period (8 s). The result showed that the higher the fat content the smaller the foam stability. Mbah et al. (1981) reported that low ash level is desirable in colour stability of beer.

The sensory evaluation by the panel members for the various attributes such as colour, taste, flavour, mouthful, and overall acceptability are shown in Table 3. For taste, flavour, colour and mouthfeel, there were no significant differences ($P < 0.05$) between 'utasi' flavoured beer and the control (hopped beer). Mouthfeel and taste increased in ratings in hopped beer and utasi beer samples more than other beer samples. For overall acceptability, 'utasi' beer was most preferred but did not show any significant difference ($P < 0.05$) from the control. The colour acceptance decreased from 'utasi' beer (8.30), down to bitter leaf powder beer (7.30), bitter leaf extract beer (7.20) and bitter kola powder beer (7.0). The decrease in average mean score in colour may be due to the light greenish colour of the vegetable extract imparted into the products whereas the bitter kola powder beer was almost colourless.

Similarly, the average mean scores of the flavour decreased from 8.60 in 'utasi' flavoured beer to 6.60 in bitter kola powder flavoured beer. The decrease in the mean scores may be as a result of the leafy flavour of the vegetable extract which was more pleasant than the bland flavour of the bitter kola extract beer. The taste mean scores in the samples generally decreased from 8.30 in 'utasi' flavoured beer to 6.90 in bitter kola powder flavoured beer. The decrease was noted to be significant ($P < 0.05$). The reason may be due to the same reason attributed to that of flavour above. The mean scores of the overall acceptability of the beer samples decreased from 7.60 in 'utasi' beer to 7.00 in bitter kola powder flavoured beer.

Conclusion

Sorghum beer was brewed using extracts of bitter vegetables and bitter kola to impact bitter taste and flavour as substitutes for hops used for beer production. The vegetables used as substitute for hop imparted the desired bitter taste and flavour in the beer samples (fig 1).

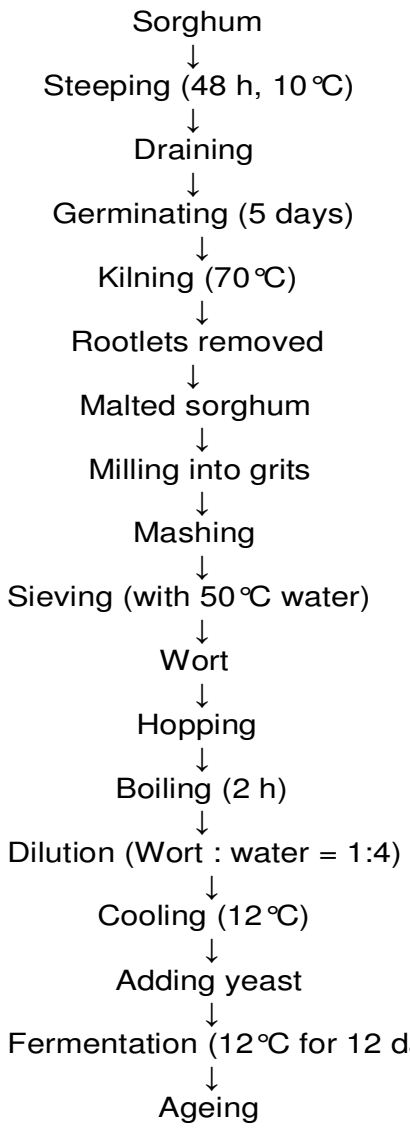

Figure 1. Flow diagram of sorghum beer production (Withy and Lodge, 1985; Sueeri, 1991).

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