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Effect of *Vernonia amygdalina* (bitter leaf) extract on brewing qualities and amino acid profiles of stout drinks from sorghum and barley malts

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Abstract

The brewing qualities and amino acid profiles of barley and sorghum-derived stout drinks produced with a hop substitute, 'bitter leaf' (*Vernonia amygdalina*) extract and residue (BLE & BLR), were analysed. The alcoholic contents, original gravities and apparent extracts of the stout drinks were essentially the same as those of a commercial stout. Seventeen free amino acids were found in the stout samples. Isoleucine (2.91 mg/100 ml), leucine (3.74 mg/100 ml), alanine (2.16 mg/100 ml), phenylalanine (3.18 mg/100 ml), tyrosine (2.56 mg/100 ml) histidine (2.05 mg/100 ml), glutamine (2.20 mg/100 ml) and proline (7.0 mg/100 ml) were the major free amino acids. All stout samples were rated the same as a commercial barley stout in terms of flavour, bitterness, colour and foam formation. The stout samples had a slightly sweet and bitter taste which was probably due to the high contents of bitter amino acids (isoleucine, leucine, histidine) and proline.

1. Introduction

Stout is a beverage obtained from the alcoholic fermentation of roasted and unroasted barley malt to which hops have been added (Jasper, & Phillip, 1974). The high cost of importing conventional brewing ingredients into Nigeria, where they cannot be produced, has led to the adoption of locally available products in their place. In particular, sorghum is now well established as a substitute for barley in Nigeria, where it is used in, both malted and unmalted forms. Also, studies (Ogundiwin & Ilori, 1991; Okoh, Babalola, & Ilori, 1995) have reported the possibility of using locally available substitutes instead of hops in beer production. One possible hop substitute is *Vernonia amygdalina*, known as 'bitter leaf', which is widely grown in Nigeria as a food vegetable and resembles the hop not only in its bitter flavour but also in its antimicrobial properties (Okoh et al., 1995).

Malted sorghum has hitherto been reported to be suitable for lager beer production (Agu, 1995; Okafor, & Aniche, 1980; Skinner, 1976). The use of malted sorghum with bitter leaf in the development of stout has been reported (Ogundiwin, & Ilori, 1991). Other references to the use of bitter leaf in other alcoholic and non-alcoholic drinks are scanty. The effective utilization of bitter leaf as a hop substitute in alcoholic and non-alcoholic drinks requires detailed information of their influence on various qualities of these drinks.

This study examines the effect of bitter leaf extract (BLE) and residue (BLR) on some brewing qualities and amino acid profiles of sorghum-derived stout drinks. Also, comparative assessments (sensory evaluation) of laboratory brewed stout drinks and a commercial stout are reported.

2. Materials and methods

A brown sorghum (*Sorghum bicolor* (L.) Moench) variety denoted 5K 5912 and barley (*Hordeum vulgare* L.) variety Georgie were obtained from the Seed Production Unit of the Institute of Agricultural Research, Zaria, Nigeria. All samples had germination energies $<98\%$. Both samples of grains were steeped (500 g grains in 1000 ml water) for 24 h, drained, air-rested

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for 4 h and then germinated at 25°C for 6 days (Lasekan, 1993). Kilning was done at 55°C for 24 h (Lasekan, Idowu, & Lasekan, 1995).

Bitter leaf juice was extracted from a bitter leaf plant (*Vernonia amygdalina*) (Ogundiwin, & Ilori, 1991) which was obtained from the University of Agriculture, Research Farm. The plant, a perennial which belongs to the family Compositae, is widely cultivated as a vegetable and used for medicinal purposes (Hutchinson, & Dalziel, 1963). Baker's yeast was purchased from a local market.

2.1. Wort production from sorghum and barley malts

Sorghum and barley malts (500 g) were medium-roasted (200°C, 4 h) separately to give a brownish endosperm colour (Lasekan, Lasekan, & Idowu, 1997). Roasted malts (50 g) and unroasted malts (200 g) were milled with a roller mill to a particle size of about 600 μm (Ilori, Akingbala, Oguntimein, & Ogundiwin, 1991). Each milled sample was mashed using the conventional mashing profile of barley malt (Ilori et al., 1991). After saccharification, filtration was carried out by pouring the mash onto a 200-μm mash screen to obtain about 1.2 l of wort. The wort was further clarified by centrifugation for 15 min at 16,000 rpm (Centrifuge type UJE, No. 60749. manufactured by PMT, Tomson, Zoetermeer, Holland). Sucrose (18% w/v) was added to the wort to increase the original gravity to 1.0582. Caramel (20% v/v), prepared from 1 kg of granulated sugar (Ogundiwin, & Ilori, 1991), was added to the wort to give a colour value of 21.0°EBC.

2.2. Wort boiling and addition of hop substitute (bitter leaf)

Each of the wort (1.2 l) samples (sorghum and barley) was flavoured with 2.5% v/v bitter leaf extract (BLE) and 0.17% w/v, bitter leaf residue (BLR) (Ogundiwin, & Ilori, 1991) and boiled for 2 h. The dark brown worts were cooled to 20°C and pitched with dried top fermenting yeast (Baker's yeast) at the rate of 2.8 g/l (Briggs, Wadeson, Staham, & Taylor, 1986), and allowed to ferment at room temperature (30 ± 2°C) for 5 days. After fermentation, the stouts were chilled to about 3°C, decanted and filtered by sifting, using a 15-μm mesh screen to remove the yeast cells.

2.3. Analysis of the wort and stout

The wort and stout samples were analysed for pH, titratable acidity, colour, iodine reaction, specific gravity and alcohol following the Institute of Brewing, IOB (1977) and AOAC (1980) recommendations. Final attenuation and saccharification time were determined as described by Van Meel (1984). The apparent degree of fermentation was calculated as described by Hough, Briggs, and Steven (1971).

Free amino acids of the samples were extracted and purified through on Amberlite IR column according to Kim, Kato, Okitani, and Hayase (1982). The contents and composition of free amino acids were analysed using an automatic amino acid analyser (LKB 4150 alpha amino acid analyzer, LKB, Bromma, Sweden). Under the analytical conditions, glutamine and threonine were coeluted at the retention time of 26.69 min. To determine the contents of glutamine and threonine, the purified free amino acid sample was hydrolyzed with 2 N HCl at 100°C for 3 h. This hydrolysis converts all the glutamine to glutamic acid (Kim et al., 1997). The glutamine content was calculated by the difference in peak area of glutamic acid before and after hydrolysis.

2.4. Sensory evaluation

A total of 10 tasters, who were regular stout drinkers, evaluated the drinks for comparative analyses. The parameters used for sensory evaluation were flavour, bitterness, colour and foam formation, and the scores were based on a five-point rating scale (5 = extremely better than *R* (commercial barley stout); 4 = better than *R*; 3 = no difference; 2 = slightly inferior to *R*; and 1 = inferior to *R*).

2.5. Statistics

Each reported value is the mean of duplicate or triplicate measurements, and data were subjected to analysis of variance and Duncan's multiple range tests were used to establish significant differences ($P = 0.05$) between means (Steel, & Torrie, 1960).

3. Results and discussion

The properties of the sorghum- and barley-derived worts are shown in Table 1. Barley-derived worts gave higher values for colour (20°EBC), pH (5.5), extract (11°P) and attenuation limit (80.3%). Also, the wort analysis shows that saccharification was very low and incomplete in sorghum wort (Table 1). There was complete saccharification in barley malt. In addition wort from sorghum filtered very slowly when compared with barley wort. This might be a result of the absence of husk and the production of lower levels of endo-β-glucanase in sorghum than in barley during germination (Aisien, Palmer, & Stark, 1983).

The effects of bitter leaf extract (BLE) on stout drinks derived from sorghum and barley malts are provided in Table 2. The original gravity, the apparent extract, pH, titratable acidity, alcohol by weight and apparent degree of fermentation of the BLE-flavoured sorghum

Table 1
Properties of sorghum- and barley-derived worts[a]

Parameters	Sorghum	Barley
Colour (°EBC)	9.5	20
pH	5.4	5.5
Specific gravity (20°C/20°C)	1.042	1.042
Extract (°P)	10.5	11
Iodine colour	+ve	−ve
Attenuation limit (%)	71.0	80.3
Saccharification limit (min)	10	10
Filtration time (min)	40	23

+ve, positive, −ve, negative.
[a] Mean of determinations in triplicates.

Table 2
Properties of BLE & BLR-flavoured stout drinks from sorghum and barley malts

Parameters	Stouts		
	Sorghum	Barley	Commercial
Original gravity (°P)	14.7a	14.7a	14.8a
pH	4.1a	4.1a	4.1a
Apparent extract (°P)	2.7a	2.8a	2.8a
Alcohol (%v/v)	4.6[ab]	4.7a	4.8a
Apparent degree of fermentation (%)	81.5a	81.2a	81.1a
Titratable acidity (g/100 ml)	0.17a	0.17a	0.16a
Colour (°EBC)	21.0b	22.0b	22.0a

Means followed by the same letter along the same row are not significantly different ($P > 0.01$). BLE: 'Bitter leaf' extract (*Vernonia amydalina*). BLR: bitter leaf residue.

stout were essentially similar to those of barley stout and that of a commercial barley stout, respectively. This probably implies that bitter leaf extract (BLE) and residue (BLR) had no effect on yeast activities during fermentation. This observation is consistent with the findings of Ogundiwin and Ilori (1991) on the development of stout from sorghum malt.

Seventeen free amino acids were found in the sorghum wort and their contents greatly varied in the BLE-flavoured stout (Table 3). Isoleucine, leucine, phenylalanine, tyrosine, alanine, histidine, glutamine and proline were the major free amino acids in the stout drinks. Free amino acids play an important role in the taste of tea and vegetables (Kim, Lee, Chang, Bock, & Jung, 1997). It has been reported that isoleucine, leucine and histidine elicit a bitter taste (Belitz & Grosch, 1987). The wort and stout samples consist of appreciable amounts of these bitter amino acids (Table 3). Addition of bitter leaf extract (BLE) and residue (BLR) to sorghum wort increases the level of these bitter amino acids in the sorghum-derived stout. Other amino acids, such as lysine, threonine, arginine, aspartic acid, glutamine and serine, were significantly ($P < 0.05$) reduced in the BLE-flavoured sorghum stout. The observed reduction in the levels of these amino acids might be due to their

Table 3
Free amino acids (mg/100 ml) of sorghum wort, BLE & BLR-flavoured sorghum stout and a commercial stout

Amino acid	Sorghum stout	Sorghum barley stout	Commercial
1. Isoleucine	2.87a	2.91a	3.13a
2. Leucine	3.72a	3.74a	3.60a
3. Lysine	0.94b	0.46c	3.52a
4. Methionine	0.51a	0.48a	0.51a
5. Phenylalanine	3.18b	3.18b	3.62a
6. Tyrosine	2.54a	2.56a	2.68a
7. Threonine	1.60a	1.20b	1.64a
8. Tryptophan	0.84a	0.85a	0.91a
9. Valine	1.92a	1.94a	1.92a
10. Arginine	1.76b	1.46c	2.52a
11. Histidine	2.05a	2.18a	2.01a
12. Alanine	2.15a	2.16a	1.23b
13. Aspartic acid	1.46a	1.15b	1.40a
14. Glutamine	2.30b	2.00c	3.16a
15. Glycine	0.58b	0.58b	0.74a
16. Proline	7.0a	7.0a	8.0a
17. Serine	2.17a	1.89b	2.01a

Means in the same row with different letters are significantly different at ($P < 0.05$).

Table 4
Sensory evaluation of sorghum, barley and commercial stout samples

Stout samples	Flavour	Bitterness	Colour	Foam formation
Sorghum	3.3a	3.2a	3.1a	3.2a
Barley	3.2a	3.2a	3.1a	3.2a
Commercial	3.1a	3.1a	3.1a	3.1a

Means with the same letter along the same column are not significantly different ($P < 0.05$). [Sensory rating; 5 = extremely better than reference *R* (commercial barley stout); 4 = better than *R*; 3 = no difference; 2 = slightly inferior to *R*; and 1 = inferior to *R*].

absorption by yeast during brewing. It is unlikely that bitter leaf extract has any influence on these amino acids. Proline which reportedly has sweet and bitter tastes (Belitz, & Grosch, 1987) was high in both sorghum-derived stout and the commercial stout.

There were no significant ($P > 0.05$) differences between the sorghum stout and the commercial stout in terms of flavour, bitterness, colour and foam formation (Table 4). Also, our organoleptic test showed that all samples had a slightly sweet and bitter taste. This might be due to the appreciable levels of bitter amino acids (isoleucine, leucine, histidine) and proline in the samples.

4. Conclusion

The brewing qualities of BLE-flavoured stout drinks (sorghum and barley) were essentially similar to those of a commercial barley stout. This probably implies that bitter leaf has no effect on yeast activities during

fermentation. Addition of BLE and BLR to sorghum wort increases the levels of bitter amino acids (isoleucine, leucine and histidine) in the sorghum stout. Sensory assessment showed no significant ($P > 0.05$) differences between BLE-flavoured stout and a commercial stout in terms of flavour, bitterness, colour and foam formation. Sorghum-derived stout had a slightly sweet and bitter taste.

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