Effect of storage on the brewing properties of tropical hop substitutes

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ABSTRACT

Tropical hop substitutes from Utazi (UTZ) *Gongronema latifolium*, Bitter Cola (BTC) *Garcinia* kola bitter leaf (BTL) *Vernonia amygdalina* and a blend (1:1.41:2.89) of the three (HSB) respectively, were produced. Stability studies were carried out to predict their suitability for brewing after one to six months storage at 5±1°C and 27±1°C respectively. These were determined using the level of reduction in their á-acid, iso-á-acid, soft resin, analytical bitterness and degree of utilization levels. Result showed that there was a general reduction of between 10% to 30% in these parameters. However, the (HSB) recorded lower losses than BTC, BLF, and UTZ. Also the samples were more stable at 5±1°C than at 27±1°C. Samples treated with $Ca(0H)_2$ had lower rate of decrease in stability with percentage loses of between 5% to 15% recorded in all the samples. Pertinently, these levels of reduction were comparable to the level of losses reported in conventional temperate hops (Humulus lupulus) stored under similar conditions. Therefore, the tropical hop substitutes when stored at 5±1°C to 27±1°C still retained an acceptably high level of their hops properties that is adequate for beer brewing.

Key words: Hop substitutes, hops, á-acid, iso-á-acid analytical bitterness, brewing.

INTRODUCTION

Hops are produced from the flowers of the plant, *Humulus lupulus,* and is a major raw material used in beer brewing for imparting flavour, colour, bitterness, foam head stability and antiseptic properties, (Hough 1986). However, hop plant is a temperate crop and has not been successfully grown in tropical countries like Nigeria, hence its importation for beer brewing is imperative. According to the Federal Office of Statistics (1986) report, it cost Nigeria about 5.5 million dollars to import hops in 1985. This high cost trend could be reduced if hops substitutes can be sourced locally.

Since the Hops of commerce is bitter, some edible tropical vegetables with bettering principles have been researched into as potential hops substitutes. Gentalium (1985) reported the use of bitter leaf, *(Gongronema latifolium)* in brewing the popular Tela-beer in Ethiopia. Okafor and Anichie (1983) brewed an acceptable lager beer with Utazi leaf (*Vernonia amygdalina*). Bitter cola *(Garcinia kola)*, according to Hutchinson and Dalziel (1985), enhances flavour of local drinks when chewed while drinking them.

Okoro, (1990) and Okoro (1993) developed a tropical hops substitute blend from Utazi, bitter cola and bitter leaf combined in the ratio of 1: 1.41: 2:89 respectively. The lager beer produced using this tropical hop substitute blend (HS-Blend) was reported to be comparable and significantly not different from beers brewed with the conventional temperate hops.

The use of these tropical hop substitutes were due to their high content of alpha-acids, iso-alpha-acid and essential oils at levels comparable to those of the temperate hop substitutes, (Okafor and Anichie, 1983; Okoro, 1993). However, no storage stability studies have been conducted on these hop substitutes developed from tropical plants.

This is necessary because the alpha-acids, iso-alpha-acids and essential oils found in these tropical hop substitutes may be unstable with storage.

The aim of this work is therefore to determine the level of retention of bitterness and flavour principles in these tropical hop substitutes at different storage conditions and duration. To determine the influence of different preparations or treatments on the shelf life of these hop substitutes.

MATERIALS AND METHODS

Raw Materials Procurement

The Bitter leaf, Bitter cola and Utazi were procured fresh from Mile 12 market, Lagos. They were washed, destalked or decorticated (for bitter cola) sorted and dried at 50±2^{0}C to moisture content of 10±2% in drought air oven. After which they were milled into powder, using hammer mill (chrysty – lab mill model 8) to 0.1m diameter particle size. The powders were blended in the ratio of 1: 1.41: 2.89, utazi: bitter cola: bitter leaf respectively, as established using linear programming (Okoro, 1990; 1993). The blend was compounded into 1gm pellets using a laboratory hand screw press locally designed and fabricated.

Shelf-stability studies

Reports on the trial brewing with these samples were reported by Okoro, 1993. The four hop substitutes were utazi Pellet (UTZ). Bitter Leaf Pellet (BLT) Bitter Cola Pellet (BTC) and Hop substitutes Blend (HSB). To further improve the stability before storage, another set of the HSB was blended with 1% $Ca(OH)_2$ before palletizing it.

All the samples were vacuum packed respectively in high density polyethylene bags and stored at 27±1^{0}C and 5±1^{0}C for period ranging from 1 to 6 mth. The stability and quality changes of the samples over this storage period were monitored every two months by determining their levels of soft resin retention, á-acid retention, iso-á-acid retention, bitterness level retention and the, Degree of Hop utilization.

Soft resin determination

Ten grammes of each sample was dissolved in 10ml of hexane, thoroughly stirred and filtered (using watman No 14 filter paper). Filtrate was dried to a constant weight at 50^{0}C. The soft resin was calculated as the percentage of the original weight of sample dissolved in the hexane.

A-acid determination

To an 0.15gm of the samples was added 100ml cold methanol in a Gallenkamp shaker flask shaker. The solution was then centrifuged at 2500 = rpm for 20min and the decanted supernatant was acidified with 0.002N HCl and its absorbance at 355nm, 325nm and 275nm was determined using spectro photometer (Pye-unicam SP6-550 UV/VIS. Model) and the alpha acid calculated using AOAC (2000) methods: alpha acid (mg/L) = 73.79 (A325) – 51.56 (A355) – 19.07 (A275) where A is absorbance reading at the specified wave length.

Iso-alpha-acid determination:

15ml sample extract was acidified with 0.5ml 6N HCl and mixed with 15ml of pure iso-octane in a shaker (Gallenkamp flask shaker), 10ml of the iso-actane extract was washed with 10ml of a mixture of methanol and 4N HCl (68:32v/v). After which 5ml, of the washed iso-octane layer was diluted with 5ml of alkaline methanol (60:40 v/v methanol: 0.5N NaoH) and its absorbance read at 255nm. The iso-á-acid (mg/L) was calculated according to AOAC (2000) method.

Iso-alpha acid (mg/L) = A_{255} (96.15) + 0.4

Analytical bitterness determination

An 0.15% (w/v) solution of the respective samples were made using distilled water. The solution was boiled for 90min cooled and filtered using watman No 14 filter paper. 10ml of the water extract of each sample were acidified with 0.5ml 6N Hcl and subsequently extracted with 20ml of iso-octone in a shaker (Gallenkamp Flask Shaker). The absorbance of the iso-octane extract was determined at 275nm using a spectrophoto meter (Pye-unicam SP 6-550 UV/VIS model). The analytical bitterness was calculated according to EBC (1989) method and reported as Analytical Bitterness unit (^{0}EBU).

A275 = ^{0}EBU, were A in absorbance at 275nm.

Degree of utilization determination: The degree of utilization of the Bittening potentials in the hop substitute were calculated as:

$$\% \text{ utilization} = \frac{\text{mg/l iso-alpha-acid} \times 100}{\text{mg/l alpha-acid}}$$

RESULTS AND DISCUSSION

Results in Table 1 show that the soft resin content of all the tropical hop substitutes (THS) decreased with storage, with HSB reducing by (10-15%), UTZ (15-30%) BLF (12-19%) and BTC (10-23%) over 6 months storage. These results compares well with losses in resins reported for the conventional hops stored at 25^{0}C for 30 weeks (12-17%) by Marr (1985) and Laws (1984). The reduction in the soft resin content of hops is a common phenomenon which is associated with the oxidations depreciation of the soft resins to hard resins with storage hough (1986). However, the low percentage reduction especially, with storage at 5^{0}C show that the THS can still retain up to 70-85% of their bitterness properties hence could still function well as hop substitute for brewing after 6 months of storage.

The stability of the á-acid component of the soft resin of any given hop is very important in determining the suitability of the hop for brewing. It is the á-acid that impacts the bitterness in the beer. Results in Table 2 show that the alpha-acid content of the tropical hop substitutes (THS) were more stable at 5±1^{0}C than at 27±1^{0}C storage with reduction of (15.0%) for HSB, (21%) for UTZ, (15.41%) for BLF and 31% for BTC. However, the a-acid content of the hop substitutes blend was more stable than those present in the individual substitutes. Generally, the low stability of the á-acid is associated with that of the soft resins. This, according to Hough (1986) is due to oxidation of a-acid with storage.

The Bitterness levels of the hop substitutes samples (Table 3) reduced, with storage at both storage temperatures. However, the percentage reductions in bitterness units were observed to be lower (between 0.5% to 8%) than percentage losses in á-acid of the samples. This is consistent with the report of Gill et al. (1979) that the loss in bitterness potentials of stored hops was usually less that 50% of the reduction in its á-acid and soft resin values. This according to Hough (1986) is because some oxidation products of á-acid and B-acids are themselves bitter and that contributes to the bitterness values of hops.

The reduction in the percentage utilization of the bitterness principles in the hop substitutes with storage (Table 4), were also not as high as recorded for á-acid reduction with storage (Table 2). A net reduction in utilization of 14.89% for HSB, 11.05% UTZ, 14.30% BLF and 11.09% for BTC were observed. This is because the percentage utilization, like the bitterness level (table IV) are not only caused by the á-acid level but also by its iso- á-acid level. According to Hough 1986, the percentage utilization is measure of the extent of extraction of á-acids and its isomerization and bitterness potentials in water, wort or beer. The utilization level obtained from the HSB (34%) BTL (32%), BTC (30%) and UTZ (28%) after 6 month of storage, compares well with those reported by Laws (1983) for the conventional hops (34-37%).

There was a marked increase in the á-acid stability of tropical hop substitutes treated with $Ca(OH)_2$ before palletizing and those not treated (table V). HSB treated with Ca $(OH)_2$ and stored for six-months at 27±1^{0}C had a 12.81% reduction in á-acid level compared to the untreated HSB with 20.54% reduction in á-acid values. The same trend was observed in UTZ (26.51%: 31.65%) BLF (14.11: 21.20) BTC (21.25: 28.50) respectively. This is consistent with the use of $Ca(OH)_2$ as hop stabilizer in the conventional hop pellet production. The observed improvement in the stability of á-acids in $Ca(OH)_2$ treated pellets may be due to the formation of calcium salts of the á-acid. The Ca- á-acid salts, according to Grant (1979) are more stable to oxidation than á-acid.

Expectedly, all samples stored at 5±1^{0}C recorded more stability in all parameters than those stored at 27±1^{0}C this is consistent with the stabilization effect of cold temperature storage against oxidation changes.

Table 1:
Changes in the soft resin levels of hop substitutes with storage

Sample	Fresh samples	1 month		3 month		5 month		6 month	
	%	5±1°C %	27°±1°C	5±1°C %	27°±1°C	5±1°C %	27°±1°C	5±1°C%	27°±1°C
HSB mg/l	15.70 ± 0.21	15.65± 0.31	14.98± 0.11	15.40± 0.12	14.10 ± 0.32	15.10 ± 0.11	13.63 ± 0.09	14.77 ± 0.09	15.03 ± 0.18
% Reduction		(1.00)	(4.59)	(2.55)	(9.87)	(3.84)	(13.18)	(4.77)	(15.03)
UTZ mg/l	16.10 ± 0.24	15.88 ± 0.21	14.32 ± 0.14	15.86 ± 0.31	12.86 ± 0.24	15.25 ± 0.11	12.03 ± 0.11	14.21 ± 0.11	11.22 ± 0.11
(% Reduction)		(1.37)	(11.19)	(1.49)	(20.12)	(5.28)	(25.28)	(11.74)	(30.1)
BLF mg/l	12.84 ± 0.09	12.70± 0.22	12.20 ± 0.21	12.01 ± 0.23	11.60 ± 0.21	11.92 ± 0.09	10.68 ± 0.23	11.70 ± 0.14	10.46 ± 0.21
(% Reduction)		(1.09)	(4.98)	(6.46)	(9.66)	(7.17)	(16.82)	(8.91)	(18.54)
BTC mg/l	9.74 ± 0.11	9.22 ± 0.31	8.85 ± 0.13	9.10 ± 0.21	8.13 ± 0.08	8.25 ± 0.05	7.82 ± 0.01	9.97 ± 0.09	7.54 ± 0.22
(% Reduction)		(5.33)	(9.14)	(6.75)	(16.54)	(15.29)	(19.71)	(18.17)	(22.59)

Each value represents mean ± S.D of 3 replicate samples.

Table: 2
Acid stability of hop substitutes with storage

Samples	0 month	1 month		3 month		5 month		6 month	
		5±10C	27±10C	5±10C	27±10C	5±10c	27±10c	5±10C	27±10C
	á-acid mg/L	á-acid mg/L	á-acid mg/L	á-acid mg/L	á-acid mg/L	á-acid mg/L	á-acid mg/L	á-acid mg/L	
HSB	10.71 ± 0.01	10.68 ± 0.01	10.26 ± 0.02	10.27 ± 0.05	9.63 ± 0.02	10.11 ± 0.01	9.46 ± 0.04	9.48 ± 0.03	9.11 ± 0.01
(% Reduction)		0.28	(4.20)	(4.11)	(10.08)	(6.14)	(11.6)	(11.48)	(8.25) ± 0.02
UTZ (% Reduction)	12.81 ± 0.01	12.42 ± 0.02 (3.04)	12.12 ± 0.01 (5.39)	11.81 ± 0.04 (7.81)	10.73 ± 0.07 (16.24)	11.51 ± 0.04 (10.15)	10.05 ± 0.02 (22.10)	11.20 ± 0.02 (12.57)	9.25 ± 0.02 (27.80)
BLF (% Reduction)	8.98 ± 0.02	8.87 ± 0.04 (1.22)	8.58 ± 0.03 (4.45)	8.53 ± 0.02 (5.01)	8.00 ± 0.02 (10.91)	8.31 ± 0.067 (7.46)	. 73 ± 0.01 (13.92)	8.01 ± 0.02 (10.80)	7.04 ± 0.04 (21.60)
BLC (% Reduction)	4.94 ± 0.01	4.84 ± 0.044. (2.02)	70 ± 0.01 (4.46)	4.61 ± 0.02 (6.68)	4.23 ± 0.05 (14.57)	4.23 ± 0.02 (14.98)	4.00 ±0.01 (19.43)	4.20 ± 0.01 (14.98)	3.41 ± 0.02 (30.97)

Each value represents means ± S.D of 3 replicate samples.

Table: 3

Analytical bitterness of hop substitutes

Sample	0 month	1 month		3 month		5 month		6 month	
		5±1°C	27±1°C	5±1°C	27±1°C	5±1°C	27±1°C	5±1°C	27±1°C
HSB (°EBU)	24.51 ± 0.21	24.29 ± 0.32	24.13 ± 0.21	24.15 ± 0.24	23.62 ± 0.34	24.14 ± 0.09	23.28 ± 0.18	23.33 ± 0.09	22.55 ± 0.20
(% Reduction)		(0.89)	(1.55)	(1.42)	(3.63)	(1.51)	(5.01)	(4.81)	(8.00)
UTZ (°EBU)	26.50 ± 0.31	26.17 ± 0.22	25.91 ± 0.24	25.43 ± 0.25	24.50 ± 0.21	25.69 ± 0.24	25.69 ± 0.22	24.41 ± 0.21	22.90± 0.22
(% Reduction)		(1.32)	(1.92)	(4.04)	(7.55)	(6.84)	(6.84)	(7.89)	(13.58)
BLF (°EBU)	24.00 ± 0.25	23.70 ± 0.18	23.24 ± 0.22	23.43 ± 0.21	22.94 ± 0.21	23.50 ± 0.32	23.29 ± 0.22	22.62 ± 0.09	21.97 ± 0.18
(% Reduction)		(1.25)	(3.16)	(2.38)	(4.42)	(2.98)	(7.12)	(5.75)	(8.45)
BTC (°EBU)	15.00 ± 0.31	14.90 ± 0.21	14.78 ± 0.31	14.74 ± 0.18	14.50 ± 0.19	15.70 ± 0.12	14.36 ± 0.24	14.48 ± 0.23	14.06 ± 0.21
(% Reduction)		(0.67)	(0.67)	(0.67)	(3.33)	(2.00)	(4.27)	(3.47)	(6.27)

Each value represents means ± S.D of 3 replicate samples.

Table 4
Percentage utilization of hop substitute with storage

Sample HSB (% Utilization)	**0 month** á acid mg/L	iso-á acid (mg/L)	**1 month** á acid	iso-á acid	**3 month** á acid	iso-á acid	**5 month** á acid	iso-á acid	**6 month** á acid	iso-á acid
HSB	10.71± 0.01	5.17± 0.01	10.26± 0.02	4.91 ± 0.01	9.63± 0.02	4.02± 0.01	9.46± 0.02	3.44± 0.02	9.48± 0.03	3.03± 0.02
(% Utilization)	(48.9%)		(47.90%)		(41.74%)		(36.75%)		(34.01)	
UTZ	12.82± 0.01	4.98± 0.02	12.12± 0.01	4.46± 0.02	10.73± 0.07	3.73± 0.01	10.05± 0.02	3.04± 0.02	9.25± 0.02	2.57± 0.01
(% Utilization)	(38.85%)		(36.81%)		(34.97%)		(30.23%)		(27.80)	
BLF	8.98± 0.02	4.17± 0.01	8.58± 0.03	3.83± 0.01	8.00± 0.02	3.24± 0.01	7.73± 0.01	2.81± 0.01	7.04± 0.04	2.31± 0.02
	(47.12%)		(44.64%)		(40.45%)		(36.32%)		(32.82)	
BTC	4.94± 0.01	1.98± 0.01	4.84± 0.04	1.83± 0.03	4.24± 0.05	1.48± 0.01	4.00± 0.01	1.27± 0.01	3.41± 0.02	0.99± 0.03
(% Utilization)	(40.00%)		(37.89%)		(35.10%)		(32.73%)		(26.91)	

Each value represents means ± S.D of 3 replicate samples.

Table 5

Stability effect of $Ca(OH)_2$ treatment on hop substitutes

Samples	0 month	1month		3month		5month		6 month	
		5±1⁰C	27±1⁰C	5±1⁰C	27±1⁰C	5±1⁰C	27±1⁰C	5±1⁰C	27±1⁰C
	á acid mg/L	á acid mg/L	á acid mg/L	á acid mg/L	á acid mg/L	á acid mg/L	á acid mg/L	á mg/L	acid mg/L
HSB (% Reduction)	10.71± 0.01	9.58 ± 0.01 (10.05%)	10.26 ± 0.05 (4.20%)	8.58 ± 0.03 (17.10%)	9.63 ± 0.01 (18.50%)	8.72 ± 0.01 (19.50%)	9.36 ± 0.02 (12.50%)	8.91 ±0.03 (20.54%)	9.25 ± 0.01 (12.81%)
HSB (% Reduction)	12.82 ± 0.01	10.88 ± 0.01 (15.12%)	12.12 ± 0.03 (5.45%)	10.21± 0.01 (23.0%)	10.73± 0.01 (16.32%)	10.13± 0.01 (28.78%)	10.05 ± 0.01 (4.75%)	8.75 ± 0.01 (20.54%)	9.42 ± 0.02 (12.81%)
BLF (% Reduction)	8.98 ± 0.02	7.22 ± 0.03 (11.50%)	8.58± 0.03 (4.57%)	7.15 ± 0.01 (18.8%)	8.00 ± 0.01 (11.08%)	7.19± 0.03 (19.90%)	7.19± 0.01 (13.90%)	7.08± 0.01 (21.20%)	7.69± 0.03 (14.41%)
BTC (% Reduction)	4.94± 0.01	4.20 ± 0.01 (14.98%)	4.84± 0.01 (4.85%)	1.86 ± 0.03 (21.10%)	4.24 ± 0.02 (14.46%)	3.78± 0.03 (26.48%)	4.00± 0.01 (19.25%)	3.53± 0.01 (28.50%)	3.41± 0.01 (21.25)

Each value represents means ± S.D of 3 replicate samples.

CONCLUSION

The observed reduction in the soft resin levels á-acid levels. bitterness levels and utilization levels with storage of the tropical hop substitutes are consistent with storage changes, but their levels of reduction are similar to those recorded for the stored conventional hops especially, if treated with $Ca(OH)_2$ before palletizing and storing at 5 ± 1^0C. Essentially, tropical hop substitutes, if produced and utilized within three to six-month can yield sufficient bitterness principles when used in beer brewing. To obtain a shelf stable hop substitute from the tropics a blend of the three identified substitutes (UTZ, BFL and BTC) treated with 1% $CA(OH)_2$, palletized, vacuum packed and stored at 5 ± 1^0C and used within 6 months of storage is recommended.

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