African Journal of Food Science Research Vol. 1 (4), pp. 033-036, December, 2013. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Effect of storage on the brewing properties of tropical hop substitutes

Okoro, Casmir Chukwuemeka[1*] and Aina, J. O.[2]

[1]Department of Food Technology, Yaba College of Technology, Lagos, Nigeria.
[2]University of Ibadan, Ibadan, Nigeria.

Accepted 30 September, 2013

Tropical hop substitute 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^{O}C and 27 ± 1^{O}C, respectively. The level of reduction in their α-acid, iso-α-acid, soft resin, analytical bitterness and degree of utilization levels were determined. 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^{O}C than at 27 ± 1^{O}C. Samples treated with $Ca(OH)_2$ had lower rate of decrease instability 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. Conclusively, tropical hop substitutes stored at 5 ± 1^{O}C to 27 ± 1^{O}C can still be used for brewing even after three to six months storage.

Key words: Hop substitutes, Hops, α-acid, iso-α-acid analytical bitterness, brewing.

INTRODUCTION

The conventional hops are produced from the flowers of the plant, *Humulus lupulus*, and are a major raw material used in beer brewing for imparting flavour, colour, bitterness, foam head stability and antiseptic properties (Hough, 1980). However, hop plant is a temperate crop and cannot be 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 are bitter, some edible tropical vegetables with bittering principles have been researched into as potential hops substitutes. Gentalium (1975) 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.

The work of Okoro, (1990, 1993) showed the successful development of a tropical hops substitute from a blend of 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 α-acids, iso- α-acid and essential oils at levels comparable to those of the temperate hop substitutes (Okafor and Anichie, 1983; Okoro, 1993). However, for the successful use of these developed tropic hops substitute or their blends, the shelf stability of these products with storage has to be determined to obtain best storage conditions or duration or treatment that will improve their shelf stability in terms of retaining their brewing potentials. This is necessary because the α-acids, iso-α-acids and essential oils found in these tropical hop

*Corresponding author. E-mail: emoko102003@yahoo.com.

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 periods, as well as 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 $50 \pm 2^{O}C$ to moisture content of $10 \pm 2\%$ in drought air oven. After which they were milled into powder, using hammer mill (chrysty – lab mill model 8) to 0.1 m 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 1 g pellets using a laboratory hand screw press locally designed and fabricated.

Preparation of samples for 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 $27 \pm 1^{O}C$ and $5 \pm 1^{O}C$ for period ranging from 1 to 6 months. 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, α- acid retention, iso-α-acid retention, bitterness level retention and the degree of hop utilization.

Soft resin determination

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

α-acid determination

To a 0.15 g of the samples was added 100 ml cold methanol in a (Gallenkamp) flask shaker. The solution was then centrifuged at 2500 pm for 20 min and the decanted supernatant was acidified with 0.002 N HCl and its absorbance at 355, 325 and 275 nm was determined using spectrophotometer (Pye-unicam sp6-550 uv/vis. Model) and the α-acid calculated using AOAC (2000) and ASBC 1976 methods:

$$\alpha\text{-Acid (mg/L)} = 73.79\ (A325) – 51.56\ (A355) – 19.07\ (A275)$$

Where A is absorbance reading at the specified wave length.

Iso-α-acid determination

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

$$\text{Iso-}\alpha\text{-acid (mg/L)} = A255\ (96.15) + 0.4$$

Preparation of the vegetable water extract for analytical bitterness determination

An 0.15% (w/v) solution of the respective samples was made using distilled water. The solution was boiled for 90 min cooled and filtered using watman No 14 filter paper. 10 ml of the water extract of each sample were acidified with 0.5 ml 6 N HCl and subsequently extracted with 20 ml of iso- octane in a shaker (Gallenkamp Flask Shaker). The absorbance of the iso-octane extract was determined at 275 nm using a spectrophotometer (Pye-unicam sp 6-550 uv/vis model) . The analytical bitterness was calculated according to EBC (1975) method and reported as Analytical Bitterness unit (^{0}EBU).

$A275 = {}^{0}EBU$, where A is absorbance at 275 nm.

Degree of utilization determination

The degrees of utilization of the bitterness potentials in the hop substitute were calculated as:

$$\% \text{ Utilization} = [\text{iso-}\alpha\text{-acid (mg/L)} \times 100]/\alpha\text{-acid (mg/L)}$$

RESULTS AND DISCUSSION

Results in Table 1 show that the soft resin content of all the tropical hop substitutes (THS) decreased with storage; HSB (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 $25^{O}C$ for 30 weeks (12-17%) by Marr (1985) and Laws (1983). The reduction in the soft resin content of hops is a common phenomenon which is associated with the oxidative depreciation of the soft resins to hard resins with storage, Hough (1980). However, the low percentage reduction especially, with storage at $5^{O}C$ show that the THS can still retain up to 70-85% of their bitterness properties, and could still function well as hop substitute for brewing after 6 months of storage.

The stability of the α-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 α-acid that impacts the bitterness in the beer. Results in Table 2 show that the α-acid content of the tropical hop substitutes (THS) were more stable at $5 \pm 1^{O}C$ than at $27 \pm 1^{O}C$ storage with reduction of 15.0% for HSB, 21% for UTZ, 15.41% for BLF and 31% for BTC. However, the α-acid content of the hop substitutes blend was more stable than those present in the individual substitutes. Generally, the instability of the α-acid is associated with that of the soft

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

Samples	Soft resin levels (%)								
	Fresh samples (%)	1 month		3 months		5 months		6 months	
		5±1⁰C	27±1⁰C	5±1⁰C	27±1⁰C	5±1⁰C	27±1⁰C	5±1⁰C	27±1⁰C
HSB	15.70	15.65 (1.00)*	14.98 (4.59)	15.40 (2.55)	14.10 (9.87)	15.10 (3.84)	13.63 (13.18)	14.77 (4.77)	15.03 (15.03)
UTZ	16.10	15.88 (1.37)	14.32	15.86	12.86	15.25	12.03	14.21	11.22
BLF	12.84	12.70 (1.09)	12.20 (4.98)	12.01 (6.46)	11.60 (9.66)	11.92 (7.17)	10.68 (16.82)	11.70 (8.91)	10.46 (18.54)
BTC	9.74	9.22 (5.33)	8.85 (9.14)	9.10 (6.75)	8.13 (16.54)	8.25 (15.29)	7.82 (19.71)	9.97 (18.17)	7.54 (22.59)

HSB = Hops substitutes blend, UTZ = utazi, BLF = bitter leaf, BTC = bitter cola.
*Values in parenthesis indicate % reduction.

Table 2. α-Acid stability of hop substitutes with storage.

Samples	α-Acid stability (mg/L)								
	Fresh samples	1 month		3 month		5 month		6 month	
		5±1 ⁰C	27±1 ⁰C	5±1 ⁰C	27±1 ⁰C	5±1 ⁰c	27±1 ⁰c	5±1 ⁰C	27±1 ⁰C
HSB	10.71	10.68 (0.28)	10.26 (4.20)	10.27 (4.11)	9.63 (10.08)	10.11 (6.14)	9.46 (11.6)	9.48 (11.48)	9.11 (8.25)
UTZ	12.81	12.42 (3.04)	12.12 (5.39)	11.81 (7.81)	10.73 (16.24)	11.51 (10.15)	10.05 (22.10)	11.20 (12.57)	9.25 (27.80)
BLF	8.98	8.87 (1.22)	8.58 (4.45)	8.53 (5.01)	8.00 (10.91)	8.31 (7.46)	7.73 (13.92)	8.01 (10.80)	7.04 (21.60)
BLC	4.94	4.84 (2.02)	4.70 (4.46)	4.61 (6.68)	4.23 (14.57)	4.20 (14.98)	4.00 (19.43)	4.20 (14.98)	3.41 (30.97)

HSB = Hops substitutes blend, UTZ = utazi, BLF = bitter leaf, BTC = bitter cola. *Values in parenthesis indicate % reduction.

Table 3. Analytical bitterness of hop substitutes.

Samples	0 month	1 month		3 month		5 month		6 month	
	⁰EBU	5±1⁰C	27±1⁰C	5±1⁰C	27±1⁰C	5±1⁰C	27±1⁰C	5±1⁰C	27±1⁰C
HSB(% Reduction)	24.51	24.29 (0.89)	24.13 (1.55)	24.15 (1.42)	23.62 (3.63)	24.14 (1.51)	23.28 (5.01)	23.33 (4.81)	22.55 (8.00)
UTZ (% Reduction)	26.50	26.17 (1.32)	25.91 (1.92)	25.43 (4.04)	24.50 (7.55)	25.69 (6.84)	25.69 (6.84)	24.41 (7.89)	22.90 (13.58)
BLF (% Reduction)	24.00	23.70 (1.25)	23.24 (3.16)	23.43 (2.38)	22.94 (4.42)	23.50 (2.98)	23.29 (7.12)	22.62 (5.75)	21.97 (8.45)
BLC (% Reduction)	15.00	14.90 (0.67)	14.78 (0.67)	14.74 (0.67)	14.50 (3.33)	13.70 (2.00)	14.36 (4.27)	14.48 (3.47)	14.06 (6.27)

resins. This, according to Hough (1986), is due to oxidation of α-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 α-acid of the samples. This is consistent with the report of Gill et al. (1979) that the loss

Table 4. Percentage utilization of hop substitute with storage.

Samples	0 Month		1 Month		3 Months		5 Months		6 Months	
HSB (% Utilization)	**αacid mg/L**	**iso-αacid (mg/L)**	**α-acid mg/L**	**iso-α-acid (mg/L)**	**α-acid mg/L**	**iso-α-acid (mg/L)**	**α-acid mg/L**	**iso-α-acid (mg/L)**	**α-aid mg/L**	**iso-α-acid (mg/L)**
HSB (% Utilization)	10.71	5.17 (48.9%)	10.26	4.91 (47.90%)	9.63	4.02 (41.74%)	9.36	3.44 (36.75%)	8.91	3.03 (34.01)
UTZ (% Utilization)	12.82	4.98 (38.85%)	12.12	4.46 (36.81%)	10.73	3.73 (34.97%)	10.05	3.04 (30.23%)	9.25	2.57 (27.80)
BLF	8.48	4.17 (47.12%)	8.58	3.83 (44.64%)	8.00	3.24 (40.45%)	7.73	2.81 (36.32%)	7.04	2.31 (32.82)
BTC (% Utilization)	4.94	1.98 (40.00%)	4.84	1.83 (37.89%)	4.24	1.48 (35.10%)	4.00	1.27 (32.73%)	3.41	0.99 (26.91)

Table 5. Stability effect of $Ca(OH)_2$ treatment on hop substitutes.

Samples	0 month	1 month		3 month		5 month		6 month	
		5±1^{0}C	27±1^{0}C	5±1^{0}C	27±1^{0}C	5±1^{0}C	27±1^{0}C	5±1^{0}C	27±1^{0}C
	α-acid mg/L	**acid mg/L**	**α-acid mg/L**	**α-acid mg/L**	**α-acid mg/L**	**α-acid mg/L**	**α-acid mg/L**	**α-acid mg/L**	**α-acid mg/L**
HSB % Red. (% Reduction)	10.71	9.58 (10.05%)	10.26 (4.20%)	8.58 (17.10%)	9.63 (18.50%)	8.72 (19.50%)	9.36 (12.50%)	8.91 (20.54%)	8.91 (12.81%)
UTZ (% Reduction)	12.82	10.88 (15.12%)	10.26 (5.45%)	10.21 (23.0%)	10.73 (16.32%)	10.13 (28.78%)	10.05 (4.75%)	8.75 (20.54%)	8.91 (12.81%)
BLF (% Reduction)	8.98	7.22 (11.50%)	8.58 (4.57%)	7.15 (18.8%)	8.00 (11.08%)	7.19 (19.90%)	7.73 (13.90%)	7.08 (21.20%)	7.04 (14.41%)
BTC (% Reduction)	4.94	4.20 (14.98%)	4.84 (4.85%)	1.86 (21.10%)	4.24 (14.46%)	3.78 (26.48%)	4.00 (19.25%)	3.53 (28.50%)	3.41 (21.25)

in bitterness potentials of stored hops was usually less that 50% of the reduction in its α-acid and soft resin values. This, according to Hough (1986), is because some oxidation products of α-acid and -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 α-acid reduction with storage (Table 2). A net reduction in utilization of 14.89% for HSB, 11.05% for UTZ, 14.30% for BLF and 11.09% for BTC were observed. This is because the percentage utilization, like the bitterness level (Table 4) is not only caused by the α-acid level but also by its iso-α-acid level. According to Hough (1986), the percentage utilization is measure of the extent of extraction of α-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 α-acid stability of tropical hop substitutes treated with $Ca(OH)_2$ before palletizing and those not treated (Table 5). HSB treated with Ca $(OH)_2$ and stored for six-months at 27 ± 1^{O}C had a 12.81% reduction in α-acid level compared to the untreated HSB with 20.54% reduction in α-acid values. The same trend in reduction was observed in UTZ (26.51%), BLF (14.11%) and BTC (21.25%). 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 α-acids in $Ca(OH)_2$ treated pellets may be due to the formation of calcium salts of the α-acid. The Ca- α-acid salts, according to Grant (1979) are more stable to oxidation than α-acid.

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

Conclusion

The observed reduction in the soft resin levels, α-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^oC. 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^oC and used within 6 months of storage is recommended.

REFERENCES

American Society of Brewing Chemist (ASBC) (1976). Methods of Analysis. 7th edition. Washington D.C. pp 560-562.

AOAC (2000) Official Methods of Analysis, 17th Edition Vol II Chapter 27, p. 16.

European Brewing Convention. Analytical (EBC) (1975). Issued by the Analysis Committee of the European Convention. 3rd edition. London.

Federal Office of Statistics (FOS) (1986). The Nigerian trade summary. Federal Ministry of Commerce and Industry Abuja Nigeria.

Gentalium A (1975). Some common medicinal and poisonous plants used in Ethiopian folk medicine in: Useful plants of West Tropical Africa. Vol. 1, 2nd edition (edited by Hutchinson KJ, Dalziel MJ) Royal Botanical gardens, Kew, London pp. 503-508.

Gill R, Laws DRJ, Goldfinch P, Henley HP (1979). Storage characteristics of some hop varieties. J. Inst. Brew. 85(2): 243-247.

Grant HL (1979). Developments in the science of Hops. Master Brewers Association of the Americas 15(14): 224-228.

Hough JS (1986). Hops and Wort Treatment in: Introduction to Brewing Science and Technology Part 1 (eds Rainbow C, Fleat GES). The Inst. Brew. London pp. 28-41.

Hutchinson KJ, Dalziel MJ (1985). The useful plants of West Tropical Africa vol. 1, 2nd edition. Royal Botanic Gardens, Kew, London pp 501-508.

Laws DRJ (1983). Commercial Brewing and Storage Trials with Yeamen Hop. J. Inst. Brew. 89(2): 92-95.

Marr JF (1985). Hop Industry Committee Analysis of Hop of 1984 crop: Rep. J. Inst. Brew. 91(1): 184-191.

Okafor N, Anichie GN (1983). West African Hop Substitutes for Sorghum Lager Beer. Brew. Dist. Int. 13(1): 20-23, 31.

Okoro CC (1990). Development of Tropical Hops substitute for the Nigerian Brewing Industry. Msc Thesis, submitted at University Ibadan, Nigeria.

Okoro CE (1993). Development of Hop substitutes from Tropical Plants. NBTE J. Agric. Technolo. Maiden ed. p. 30-35.