THE POTENTIAL OF GESHO (RHAMNUS PRINOIDES L. HERIT) AS SUBSTITUTES FOR HOP (HUMULUS LUPULUS) IN BEER PRODUCTION

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ABSTRACT

Rhamnus prinoides (gesho) which is belongs to the family Rhamnaceae and order Rhamnales is a dicotyledonous angiosperm plant. In Ethiopia, the plant is utilized as a bittering agent in local alcoholic beverages. Due to its bittering substance compositions it could have the potential to be used as a raw material for alcoholic beverages. However, knowledge of its potential as a hop substitute during alcoholic beverage production is limited. The major goal of this research was to investigate the potential of R. prinoides as a raw material substitute for hops in the production of alcoholic beverages. Bittering substance of R. prinoides and physic-chemical of finished beer were done. Four beer types were identified and designated as A, B, C and D (control). The analysis of key brewing variables of the R. prinoides was as follows: total resin (15.96-16.02%), ISO-alpha acid (1.17-1.45 mg/l), alpha acid (1.44-1.92 mg/l), essential oil (3.-3.07 %), were obtained. Beer type C (7.4γ0.01%/w/w and 9.5γ0.01%/v/v) has been shown significantly (p<0.05) greater concentration of alcohol in comparison with other beer types. Real degree of fermentation value of beer type D (66.29±0.03%) was significantly (p<0.05) different compared with other beer types. Bitterness value (2.2 to 2.8) of the beer produced by R. prinoides was in the range of the values (0-100) of common hopped beer. The bitter substance compositions and key brewing variables of beer produced by R. prinoides are comparable with hop. This indicates that R. prinoides can serve as a substitute for hops in the breweries.

Keywords: Beer, Bittering agent, Hop, Rhamnus prinoides

INTRODUCTION

Plants have enormous importance with a number of potentials use for medicines and other essential compounds that have to be discovered and characterized around the world (Kaufman et al., 2006). Many unknown chemical compounds are under investigation and characterization. The discovery of these compounds can be used to substitute one raw material by another in every field of chemistry and biotechnology. Of those un-investigated plant species, gesho (Rhamnus prinoides) are widely used as a bittering agent for Ethiopian local alcoholic beverages, such as Tella, Areki, Tej and Korefe (Ashenafi, 2008). Rhamnus prinoides has the potential to be used as a substitute for hops (Humulus lupulus), which are used in brewing industry. Humulus lupulus is a potential plant that belongs to the hemp (cannabis) family and urticales order. Along with barley, water, and yeast, hops are one of the basic ingredients required to produce beer (Russell, 2003). Beer quality is significantly influenced by the quality of these raw materials. Among the raw materials used in beer production, hop (H. lupulus) plays a great role as a bittering agent. This is because they have bitter resins and essential oils, which give beer its bitterness and aroma (Kunze, 1996). Hops are grown in temperate zone, where the favorable conditions exist (Kunze, 1996) but not in a tropical region including Ethiopia. Rhamnus prinoides is one of the raw materials commonly utilized as a bittering ingredient in the production of local alcoholic beverages (Tella), it is different from hops and is primarily grown in Ethiopia. The plant is now sold in dry form in local markets in different parts of the country. Although gesho have antibacterial effects against some groups of bacteria (Berhanu, 2014), its primary function is to give the typical bitter taste to Tella (Ashenafi, 2008). The plant classified to the family Rhamnaceae and order Rhamnales, it is a dicotyledonous angiosperm plant. It is a shrub or tree which grows up to six meters. It is also grown in Cameroon, Sudan, Angola, and Eastern Africa to Southern Africa countries (Thulin, 1988). Rhamnus prinoides has many uses amongst the inhabitants of Africa. All parts of the plant are harvested and used for nutrition, medicines or religious purposes (Gebre & Singh, 2012). In Ethiopia, it is used in a manner similar to hops (Cauk, 1971). As that of hop, R. prinoides has been used as a bittering agent; serve as antiseptic agents against microbial flora rather than yeast, in coloring and flavoring of Tella and Tej (Abegaz & Peter, 1995). A naphthalene glucosidale named geshoidin has been identified as one of the ingredients, which is accountable for the bitter attribute of the plant in Tella and Tej preparation (Abegaz & Peter, 1995). In tropical countries, including Ethiopia hops are imported from abroad. Due to the growth of brewing business in tropical regions, much more money being spent on the importation of hops (Adama et al., 2011). Less attention has been given to replacing hops with a regional bittering ingredient, especially in Africa. According to early research by Okafor and Anichie (1983), the leaves of the tropical plant Grigononema latifolium (Utazi) have a lot of potential as a hop’s alternative. While the chemical characteristics of beer made with this plant were not significantly different from beer brewed with hops, there were significant organoleptic changes. Three other bitter tropical plants that are consumed as food were the subject of another investigation. These were Vernononia amygdalina (bitter leaf), Azadiractha indica (Neem) and Garcinia cola (Bitter Kola). In their work, they conclude that all have great potential as a substitute for hops (Ajobersone & Aina, 2004). In the same manner, currently, there is a need to analyze and investigate the potential of the R. prinoides as hop substitute for beer production. Rhamnus prinoides has several characteristics like an antibiotic effect, citrus, herbal aromas and flavors to the traditional agent in different alcoholic beverage, which are desirable by many brewers in beer production (Berhanu, 2014). Although R. prinoides is a potential bittering agent, it has only been used in traditional alcoholic beverages; no attempt has been undertaken to use the plant material for commercial alcoholic beverages. Hence, in this study the potential of the R. prinoides as a bittering agent for beer production in comparison with well-known bittering substance, hop was investigated. The bitter substance determination and sensory evaluation of beer produced with R. prinoides were evaluated to examine R. prinoides as a substitute for hops in brewery industry. The outcome of this work can be used as a starting point to formulate bittering substance needed for beer production, helping to reduce the amount of money spent on hop importation and generating employment opportunities for farmers and other members of the community who will be involved in gesho plantation.

MATERIALS AND METHODS

Study area

The study was conducted at North Gondar, University of Gondar molecular biology laboratory and Dashen Brewery Factory. The experiment for production of beer from gesho (Rhamnus prinoides) as hop substitute, physico-chemical analysis, beer fermentation process and sensory quality evaluation were conducted...
in the Dashen Brewery Factory Laboratory. Dashen Brewery Factory is located at 727 kilometers away from Addis Ababa, in North Gondar, North-west Ethiopia.

Raw material

The materials used to run all experiments were: Hops, Barley malt and Yeast (Saccharomyces cerevisiae) from Dashen Brewery and gesho (Rhamnus prinoides) from a local market in Gondar Town. Samples were taken at the University of Gondar department of Biotechnology molecular laboratory room for further analysis. Laboratory analysis was performed to determine the R. prinoides constituents for brewing.

Experimental work and beer sample

Analysis was done on beer sample designated as A, B, C and D to test the physical-chemical parameters. In this research, all parameters remained the same with the exception of three (A, B, and C) Rhamnus prinoides samples collected from local market and sample D normal beer fermented with a commercial hop as a control.

Sample preparation

Rhamnus prinoides leaf samples were sun dried before being crushed with a mortar and pestle. To remove the moisture content of gesho flour, it was dried in an oven at 60°C for 24 hours. The bittering compounds and other elements of R. prinoides were then identified, and it was utilized to make beer as a hop substitute.

Determination of alpha acid and beta acid determination

ISO-alpha acid determination

In order to determine ISO-alpha acids 15 milliliters of the sample extract were mixed with 15 milliliters of pure ISO-octane and the it was acidified with 0.5 milliliters of 6 N HCl. Ten milliliters of the ISO-acetone extract were washed with 10 milliliters of a 68:32 (v/v) solution of methanol and 4 N HCl. The absorbance of 5 ml of the washed ISO-octane layer was measured at 255 nm after being diluted with 5 ml of alkaline methanol (60:40, v/v methanol: 0.5 N NaOH). The (AOAC, 2000) method of analysis was used to calculate the ISO-alpha acid (mg/L). ISO-α-acid (mg/L) =A255 (96.15) +0.4

Beer production

The procedure of beer brewing was carried out using all raw materials (hop, water, Saccharomyces cerevisiae) except that of gesho instead of hop as a bittering agent. However, hop (H. lupulus var. lupulus) was used as a control using the same procedure (Kunze, 2004).

Boiling of wort

The wort was boiled and gesho was added as usual used in the beer brewing process (Kunze, 2004). It was stirred until it gets wet. The amount of the gesho used as a hop substitute was (0.5 g/L gesho), while for control (0.15 g/L hop) was used as the factory use for beer production process. The mixture of wort and gesho was mixed and boiled for 15 min to 121°C to kill all microorganisms. After that, it was allowed to cool for yeast pitching and fermentation.

Fermentation

The fermentation of sugar-laden wort carried out by the inoculation of S. cerevisiae for fermentation. The yeast was pitched into the propagation flask that containing the same type of wort for fermentation. The flask was closed and cooled to 10 to 11°C. This process kept for one day. The fermentation in the flask was checked by observing the formation of good foam. The fermenter was placed in a protected area to avoid fluctuated environmental conditions. It was placed in an area that is not exposed to direct sunlight.

Determination of physicochemical characteristics of beer

Specific gravity determination

The specific gravity of the sample was determined by 24 hourly using a digital density meter after 72 h of inoculation of yeast to the wort sugar. To identify the level of fermentation per 3 days, a sample of beer was filtered using filter aids, and specific gravity of the sample was determined using density meter at 20°C until the extract arrives at 3 and below with the correlation table (EBC, 2008). At the end of fermentation, the specific gravity of the bicarbonate apparent extract, alcohol, and real extract was determined using pyknometer at 20°C after distillation.

Determination of real extract

Real extract was determined by conversion of the specific gravity of the residue to the corresponding real extract content, Er as % plato (Rosendal and Schmidt,1987).

\[
\text{Er (\% Plato)} = \frac{460.234 +662.649 \text{SG}_{\text{IR}}-202.414 \text{SG}_{\text{IR}}^2}{2}
\]

Determination of apparent extract

Apparent extract was determined by conversion of the specific gravity of the filtered beer to the corresponding apparent extract content, Ea as % plato (Rosendal & Schmidt,1987).

\[
\text{Ea (\% Plato)} = \frac{460.234+662.649 \text{SG}_{\text{IR}}-202.414 \text{SG}_{\text{IR}}^2}{2}
\]

Determination of real degree of fermentation

Real degree of fermentation was calculated with the formula, RDF=100× [0.6655×A2+0.6655×A1+Er×%)

Where, A=alcohol, % (v/v)

\[
\text{Er} = \text{real extract, Plato (EBC, 2000)}
\]

Determination of alcohol content

The alcohol content was determined using distillation by direct heating and determining the alcohol % (w/w) from the distillate specific gravity, the alcohol % (v/v) content was determined from the specific gravity of the filtered beer and alcohol % (w/w) (EBC, 2000).
Determination of pH

Two hundred ml of beer samples was filtered by filter paper and excess carbon dioxide was removed by shaking to prevent unstable pH reading. The electrode of the pH meter was inserted into the beer sample and the reading on the screen of the pH meter was observed and recorded (EBC, 2000).

Determination of bitterness in beer

The beer sample was re-filtered and 100 ml was taken after adding 3 drops of octanol. Ten ml of the sample and 1 ml of HCl together with 20 ml ISO-octane was mixed and then shaken with platform shaker until maximum extraction was achieved. Absorbance of ISO-octane layer in 10 mm Cuvette at 275 nm was measured using pure ISO-octane in the reference Cuvette (EBC, 2000). Bitterness (BU) = A * 50

Where A = Absorbance at 275mm

Determination of carbon dioxide in beer

The carbon dioxide content of the beer was determined using titration method. Ten ml NaOH was poured into a 250 ml flask and 200 ml of beer sample was added and it was inserted into the right side of the sample point outlet with the addition of 10ml H2SO4. On the other hand, 25 ml barium hydroxide was taken and inserted in the left side of the apparatus and the two fork tubes was connected with hoses to allow air circulation. After completion of the air circulation 3 drops of phenophtaline indicator were added to allow titration. After titration was stopped 3 drops of HCl were poured into a 250 ml flask and titrated with HCl the excess barium hydroxide up to the end point (EBC, 2000).

\[
\text{CO}_2 + 2\text{NaOH} = \text{Na}_2\text{CO}_3 + \text{H}_2\text{O}
\]

\[
\text{Na}_2\text{CO}_3 + \text{H}_2\text{SO}_4 = \text{Na}_2\text{SO}_4 + \text{CO}_2 + \text{H}_2\text{O}
\]

\[
\text{CO}_3^2^- + \text{Ba}^{2+} = \text{BaCO}_3
\]

\[
\% \text{CO}_2/\text{litre} = 100 (D - E)/0.0022 \times \text{C/A} + \text{B}
\]

Where A, content of bottle or cans in ml
B, NaOH added in ml
C, sample volume in ml
D, 0.100m HCl used for the titration of 25.00 ml Ba(OH)2 (blank value) in ml
E, 0.100m HCl used for the titration sample in ml

### Table 1: Assessment of bitterness of substances in R. prinoides

| Samples | Total resin | Hard resin | Soft resin | Alpha acids | Iso-alpha Acids | Essential oil | Beta acids |
|---------|-------------|------------|------------|-------------|----------------|---------------|------------|
| A       | 16.02±0.04a | 9.96±0.02a | 6.24±0.26a | 1.44±0.01a  | 1.17±0.05a     | 3.04±0.05a    | 2.18±0.02a |
| B       | 16.42±0.05a | 9.72±0.26a | 6.39±0.24a | 1.66±0.12a  | 1.19±0.02a     | 3.09±0.01a    | 2.14±0.11a |
| C       | 15.96±0.02b | 9.94±0.04a | 6.02±0.09a | 1.92±0.11b  | 1.45±0.47a     | 3.07±0.06b    | 2.07±0.11b |

*Values are the means of triplicate determinations ± standard deviation; values within the same column followed by different superscripts are significantly different (p < 0.05).

### Analysis of specific gravity of beer that produced using R. prinoides

The specific gravity of the fermentation test for beer brewing with R. prinoides was shown in Table 2. The degree of extract decrease along with fermentation date was used for the immediate control of the fermentation process. When the extract reaches three and below that the fermentation process stopped theoretically. According to this finding, beer type designated as B (decrease from 13.46 to 3.02) and D (decrease from 11.68 to 3.05) has relatively good fermentation performance in comparison with other beer type. All beer types at day eighteen shows a similar extract compared with extract at day fifteen, thus the fermentation process stopped at day eighty.

### Table 2: Specific gravity (in degree Plato) of beer samples produced by R. prinoides and hop

| Date of fermentation | Beer sample |
|----------------------|-------------|
|                      | Sg (°P) A   | Sg (°P) B   | Sg (°P) C   | Sg (°P) D (control) |
| 3                    | 10.42       | 10.68       | 11.02       | 10.64 |
| 6                    | 7.38        | 7.50        | 8.46        | 7.38  |
| 9                    | 5.78        | 4.98        | 5.30        | 5.4   |
| 12                   | 3.75        | 3.32        | 5.15        | 3.75  |
| 15                   | 3.07        | 3.02        | 4.8         | 3.05  |
| 18                   | ++          | ++          | ++          | ++    |

**++=Fermentation stopped, Sg= Specific gravity**

### Physicochemical characteristics of beer produced by Rhamnus prinoides

Analysis of the alcohol content of beer produced by R. prinoides

Ethanol is a major end product of beer fermentation. It forms part of the end byproducts of work fermentation. Analysis of results showed that beer type C (7.4±0.01%/w/w and 9.5±0.01%/v/v) has been shown significantly (p<0.05) greater concentration of alcohol in comparison with beer A (6.6±0.01%/w/w and 8.5±0.02%v/v), beer B (6.8±0.01% w/w and 8.4±0.01%v/v) has been shown low (p>0.05) alcohol content compared with other beer types. Beer produced with hop has been shown lower (p<0.05) amount of alcohol (5.2±0.01% w/w and 6.6±0.01 v/v) than the rest type of beers.

### Table 3: Alcohol content of beer produced by R. prinoides

| Beer type | Alcohol level (% w/w) | Alcohol level (% v/v) |
|-----------|------------------------|-----------------------|
| A         | 6.6±0.01                | 8.5±0.02               |
| B         | 6.8±0.01                | 8.4±0.01               |
| C         | 7.4±0.01                | 9.5±0.01               |
| D (control) | 5.2±0.01               | 6.6±0.01               |

*Values are the means of triplicate determinations ± standard deviation; values within the same column followed by different superscripts are significantly different (p < 0.05).
Analysis of original extract, apparent extract, real extract and the real degree of fermentation of beer produced by *Rhamnus prinoides*

The value of the original extract of beer type B (21.60±0.01) and beer type C (21.62 ±0.01) were statistically (p>0.05) similar and are different with the other beer types evaluated. Beer type B resulted in relatively (p<0.05) lower original extract (15.70±0.01) as compared to the other beer types such as A (20.31±0.01), C (21.77±0.01) and D (18.88±0.01) beers. Beer type A has been statistically (p<0.05) greater apparent extract (4.80±0.01%) in comparison with other beer types. Beer D had been statistically (p<0.05) less apparent extract (4.20±0.01%) than the other beer types. The values of total resin obtained (15.96±0.01) were used as an indicator of the progress of fermentation starting values at the wort stage, they were significantly lower. According to this work, the primary elements of hop resins, alpha acids, beta acids, and the products of their transformation are known to contribute to beer bitterness (Kunze, 1996). The primary elements of hop resins, alpha-acids, beta-acids, and the products of their transformation are known to contribute to beer bitterness (Kunze, 1996). The oils in hop contain fatty acids and esters, which impart the aroma and flavor of beer. The oil component of *H. lupulus* ranges from 0.03-3% (Kunze, 2003). In this study, a significant amount of (3.00-3.07%) oil content was obtained and it indicates *R. prinoides* can be a source of flavor in production of commercial beer. The results obtained from *R. prinoides* for alpha-acids, beta-acids, and essential oil was found to be within the range of dry hops (Hieronymus, 2012). Thus, the value of the plant extract from the analysis performed can be said good since there were similarities in the properties of the standard commercial hops and the *R. prinoides* properties. By measuring the wort's specific gravity as fermentation occurred, the breakdown of the wort components was used as an indicator of the progress of fermentation process. Comparing the post-fermentation specific gravity measurements to their starting values at the wort stage were significantly lower. According to this experiment, the profile of the *R. prinoides* beer was comparable to the profile of the hopped (control) beer (Rourke, 2002). For quality assurance programs and legal reporting requirements, the examination of beer's alcohol content plays a significant role in the brewing process (Kunze, 1996). The alcohol concentrations in this investigation ranged from (5.2 to 6.7%) w/w and (6.6 to 9.5%) v/v. The alcohol concentration of the samples used in this investigation, both w/w and v/v, was within the range for strong beer. The alcohol content of beer in this study (7.4% w/w and 9.5% v/v) shows that more sugar was fermented in these beer samples than in other samples. The alcohol levels were comparable to those that reported by Okafor and Anichie (1983).

**Table 4** Analysis of vicinal diketones, bitterness, total acidity, carbon dioxide, and pH of beer produced by *R. prinoides*

| Beer type | Vicinal diketone (mg/l) | Bitterness unit | Total acidity | Carbon dioxide | pH     |
|-----------|------------------------|----------------|--------------|----------------|-------|
| A         | 0.25±0.01              | 2.20±0.15      | 0.62±0.01    | 4.20±0.01      | 4.70±0.02 |
| B         | 0.24±0.01              | 2.40±0.05      | 0.61±0.02    | 4.30±0.05      | 4.40±0.05 |
| C         | 0.26±0.01              | 2.80±0.17      | 0.59±0.01    | 4.30±0.05      | 4.70±0.02 |
| D (control)| 0.21±0.01             | 25.00±0.05     | 0.42±0.01    | 4.20±0.01      | 4.70±0.02 |

Based on the mean value obtained the total acidity of beer type D (0.24±0.01) was less than (p<0.05) total acidity compared with other beer types. All other three beer types have been shown statistically (p>0.05) similar values of total acidity. Based on the mean value of CO₂ obtained in this study, beer type D (0.25±0.01) had been statistically (p<0.05) greater value of carbon dioxide than the other types of beer. Beer type A (4.2±0.01) and beer type B (4.3±0.005) have been shown statistically (p<0.05) similar pH values. The result obtained by beer type D (4.7±0.02) has been significantly (p<0.05) higher pH than compared with other beer types.

**Table 5** Analysis of vicinal diketones, bitterness, total acidity, carbon dioxide and pH of beer produced by *R. prinoides*

| Beer type | Vicinal diketone (mg/l) | Bitterness unit | Total acidity | Carbon dioxide | pH     |
|-----------|------------------------|----------------|--------------|----------------|-------|
| A         | 0.25±0.01              | 2.20±0.15      | 0.62±0.01    | 4.20±0.01      | 4.70±0.02 |
| B         | 0.24±0.01              | 2.40±0.05      | 0.61±0.02    | 4.30±0.05      | 4.40±0.05 |
| C         | 0.26±0.01              | 2.80±0.17      | 0.59±0.01    | 4.30±0.05      | 4.70±0.02 |
| D (control)| 0.21±0.01             | 25.00±0.05     | 0.42±0.01    | 4.20±0.01      | 4.70±0.02 |

Detection of microbial contaminant in beer produced by *Rhamnus prinoides*

Availability of microorganisms in beer produced by *R. prinoides* was evaluated using a standard micro-cultural system. Beer samples were spored on universal beer agar medium and incubated for seven days at 25°C. Microorganism such as molds, wort bacteria and lactic acid bacteria were not observed on cultured beer after seven days of incubation.

**DISCUSSION**

The research aimed at determining the bittering capacity of gesho (*Rhamnus prinoides*) as a substitute for hops (*Humulus lupulus*) used in brewing of beers, the research attempted to determine the key variances identified as necessary in hops. Characterization of the physicochemical characteristics of the *R. prinoides* extract was done in this investigation to identify any bitter components. The content and quality of the raw materials used are the main determinants of beer quality. The primary brewing ingredient used as a bitterness agent is hop. For the quality of the beer and the cost-effectiveness of the brewing process, its chemical composition is very important. In this study important physicochemical characteristics of beer analysis have been investigated to know the bitterness potential of *R. prinoides* on sensory quality of beer.

The values of total resin obtained (15.96-16.02%) evaluated for *R. prinoides* were comparable with *Humulus lupulus* (16.53%) used as a known bittering agent in commercial beer (Kunze, 1996). In this study, the quantity of total resins of *R. prinoides* was comparable with other bittering hop substitutes such as *Vernonia amygdalina* and commercial hop (*Adama et al., 2011; Kunze, 1996*). The values of total resin, soft resin and hard resin components of *R. prinoides* were less than the values obtained by Berhanu (2014), who studied the bittering and antimicrobial role of this plant But, the value of oil content was higher than the value recorded by Berhanu (2014), it supports the ideas of hop constituents are place of cultivation dependent.

The highest (p<0.05) apparent degree of fermentation value (79.72±0.005) was observed by beer type D. Beer type C has been shown statistically (p<0.05) lower apparent degree of fermentation (75.91±0.01) than beer type A (77.50±0.01) and beer type B (77.60±0.01). The highest (p<0.05) real extract value was observed (8.01±0.01) by the beer type A in this investigation. The value of real extract (5.47±0.01) observed in beer type D was statistically (p<0.05) lower than the rest beer types. The highest real degree of fermentation value (66.29±0.03) recorded by beer D was significantly (p<0.05) different compared with other beer types (beer A 64.93±0.01, beer B 63.22±0.01) and (beer C 62.01±0.005). The lowest result recorded was by beer type C in this study.

**Table 4** Analysis of vicinal diketones, bitterness, total acidity, carbon dioxide and pH of beer produced by *R. prinoides*
The real degree of fermentation capacity of beer A studied in this experiment agreed with hopped beer, the apparent degree of fermentation of all beer types was lower than the hopped (control) beer. For extra strong beer, the minimum standard values for apparent extract and true extract are 2.5% and 4.4%, respectively (ES842, 2012). R. prinoides produced beer with a minimum apparent and true extract of 4.46% and 7.62%, respectively. The apparent and real extract percentages for the hopped (control) beer were 4.2% and 5.47%, respectively. Thus, both the apparent and real extract values found in this investigation were significantly higher than the required minimum levels for extremely strong beer. This study demonstrates that the R. prinoides-produced beer exhibits a very good fermentation process for the production of industrially commercialized beer.

The pH values of beer produced were within the range of 4.2 and 4.7. The pH values of beers were within the standard value (3.6 to 4.8) of (ES830, 2012). The pH has a significant effect on the quality of beer (Kunze, 1996). The pH can reduce the possible contamination effect of beer. The total acidity value of beer types A (0.62/10 ml) and beer B (0.61/10ml) was similar to the total acidity value given by Okafor and Anichie (1983) for tropical hop substitute beer. In contrast, the total acidity value for hopped (control) beer was lower than the value recorded by Okafor and Anichie (1983). This indicates that hopped beer has a lower acid level than beer brewed with R. prinoides.

Hops are generally responsible for the bitterness of beers, in addition to hops polyphenol can also impart beer bitterness (Kunze, 1996). It typically results from the main bitterness component of hops, ISO-acids, isomerizing to -acids during wort boiling. Bitterness should be monitored and closely managed to preserve uniformity in quality. IBU ratings for various types of beer typically range from 0 to 100 IBU (Kunze, 1996). The R. prinoides produced beers in this research were significantly less than to the hopped (control). In this investigation, the beer C was shown to have more bitterness compared with other beer type produced by R. prinoides. The degree of bitterness of beer in this investigation was within the range of 2.2 to 25.0 IBU. All beers produced were within the range of the beer types stated by Kunze, 1996.

Vicinal diketones (VDK) provide beer a sweet flavor if their concentration exceeds the limit value and give it a butty aroma (Fix, 1993). Beer made from R. prinoides had a higher VDK concentration than the controlled beer. In general, the beers made by R. prinoides in this investigation contain VDK values that are relatively higher than the reference value (0.15 mg/L) (ES843, 2012). Generally, the amount of releasing carbon dioxide (CO2) during fermentation is a direct indicator of a good fermentation activity during the brewing process (Kunze, 1996). The CO2-content of beer is one of its most important quality criteria. In this finding, carbon dioxide concentrations of all beer types were shown by far less than the specification in good beers (4.7g/L–5.2g/L) (Fix, 1993). The microbiological profile of beer made from R. prinoides need to be determined in order to assess the quality of beer. It is commonly recognized that beer contaminants could spoil beer, which lowers its quality. Usually, inadequate cleanliness and raw materials cause wort germs to grow in the fermenting vessel. Sterilized wort, pure yeast, and the sterilized and cooled vessel were all free of contamination in this investigation. This might be as a result of R. prinoides's antimicrobial properties (Berhanu, 2014).

CONCLUSION

Rhamnus prinoides can be used as a bittering agent in alcoholic beverages production. The goal of this research was to find R. prinoides bittering agents that could be used to make commercial beer. The beer produced using R. prinoides was comparable to that produced with hops as a bittering agent. According to the findings of this investigation, R. prinoides can be used as a bittering agent as a substitute of hops. The study result can be utilized as a starting point for formulating this bittering substance for commercial beer brewing, reducing the amount of money spent on hops and providing job opportunities for farmers and other members of society who grow gesho plants.

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Conflict of Interest: The authors have no conflict of interest to declare.

REFERENCES

Abegaz, B. M., & Peter, M. G. (1995). Emodin and emodinanthrone rhamnoides acetates from fruits of Rhamnus prinoides. Phytochemistry, 39(6), 1411–1414. https://doi.org/10.1016/0031-1820(95)00093-m

Adama, K. K., Oferato, A. A., Dika, S.I. & Sheda. (2011). Bitterleaf as local substitute for hops in the Nigerian brewing industry. Scholars Reseach Library, 3(4): 388-397.

Adenuga, W., Olaleye, O. N., & Adepoju, P. A. (2010). Utilization of bitter vegetable leaves (Gongronema latifolium, Vernonioa amygdalina) and Garcinia kola extracts as substitutes for hops in sorghum beer production. African Journal of Biotechnology, 9(51): 8819-8823.

Ajbersonse, P.E., & Aina, J.O. (2004). Potential African Substances for Hops in Tropical Beer Brewing. J. Fed. Tech. Afr, 9(1):13-16.

AOAC. (2000). Official Methods of Food Analysis. 17th Edition, Chapter 27, 2:16. 

Ashenafi, M. (2008). Review Article: A Review on the Microbiology of Indigenous Fermented Foods and Beverages of Ethiopia. Ethiopian Journal of Biological Sciences, 5(2), https://doi.org/10.4314/ejbs.v5i2.90036

Berhanu, A. (2014). Microbial profile of Tella and the role of gesho (Rhamnus prinoides) as bittering and antimicrobial agent in traditional Tella (Beer) production. Int Food Res J, 21: 357-365.

Caulk, R. A. (1971). Economic History of Ethiopia, 1800–1935 by Richard Panckhurst Addis Ababa, Haile Sellassie I University, 1968. Pp. 772. The Journal of Modern African Studies, 9(3), 490–492. https://doi.org/10.1017/s0022278x00025301

EBC Analytica, John. W. (2000). Methods for the determination of alcohol. European Beer Convention, Journal of the institution of brewing, 66(3):143-150.

EBC Analytica, Banforth, Charless W. (2008). Methods for measurement of beer and haze in beer. European Beer Convention Chemistry, 66(3): 143-150

Ethiopian Standard 828, (2012). Standard of real extract value of beer. ES test method, 842.

Ethiopian Standard 828, (2012). Standard pH value of beer. ES test method, 830.

Ethiopian Standard 828, (2012). Standard VDK value of beer. ES test method, 843.

Fix, G. (1993). Principles of brewing science. Brewers Publications, 2:23-24.

Gebre, A., & Singh Chandravanshi, B. (2012). Levels of essential and non-essential metals in Rhamnus prinoides (Gesho) cultivated in Ethiopia. Bulletin of the Chemical Society of Ethiopia, 26(3). https://doi:10.4314/bces.v26i3.2

Hieronymus, S. (2012). For the love of hops: The practical guide to aroma, bitterness, and the culture of hops. Boulder, CO: Brewers Publications.

Kaufman, P., Brielmann, H., Cseke, I., Setzer, W., & Kirakosyan, A. (2006). Phytochemicals. Natural Products from Plants, 2nd Edition, 1–49. https://doi.org/10.1201/9781420044725.ch1

Kunze, W. (1996). Technology Brewing and malting; international edition. English translation of the 7th revised edition of Technologie Brauer und malzer. ISBN 3-921 690-34-X. VLB Berlin, Verlagsabteilung.

Kunze, W. (2004). Technology brewing and malting, 3rd completely updated edition, VLB Berlin, Germany.

Kunze, W. (2003). “Technology of brewing and malting, 3rd edition”, ISBN 3-921 690-49-8, VLB-Berlin.

Russell, I. (2003). Brewing Yeast and Fermentation: Chris Boulton and David Quinn; Journal of the Institute of Brewing, 109(2), 161–161. https://doi.org/10.1002/2050-0416.2003.d00148.x

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

Rourke, O.T. (1994). The Requirement of Beer Stabilization, Brewers’ Guardian, 123 (8).

Thulin, R. (1988). In Flora of Ethiopia, Vol. 3, Hedberg, I.; Addis Ababa and Asmara, Ethiopia, and upsala, Sweden.

Rosenfeld, I & Schmidt, F. (1987). The alcohol table for beer analysis and polynomials for alcohol and extract. Journal of institute of brewing, 93:373-377.

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