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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-.0-3.07 %), were obtained. Beer type C (7.4±0.01%w/w and 9.5±0.01%v/v) has been shown significantly ($p \leq 0.05$) 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 ($p \leq 0.05$) 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 material 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 climate 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 Cameron, 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 **(Caulk, 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 glucosidal 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 *Grongonema latifoium* (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 *Vernonica amygdalina* (bitter leaf), *Azadiracbta indica* (Neem) and *Garcinia cola* (Bitter Kola). In their work, they conclude that all have great potential as a substitute for hops **(Ajebersone &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 t 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 (*Sacharomyces cervisiae*) 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 physic-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.

Determinations of bittering compositions of *Rhamnus prinoides*

Total resin determination

Twenty gram of the sample was dissolved in 100 ml of cold methanol in a conical bottom flask and the mixture was vigorously agitated by swirling the flask. The solution was then filtered. The filtrate containing the resin was dried and the total resin was calculated as a percentage of the original sample weight **(Adama *et al.*, 2011)**.

Soft resin and hard resin determination

Ten gram of each sample was properly mixed with 10 ml of hexane before being filtered (using watman filter paper). The filtrate was heated to 50ºC and dried to a constant weight. The amount of the original sample weight that was dissolved in the hexane was used to compute the amount of soft resin. Soft resin was subtracted from total resin to get the hard resin **(Adama *et al.*, 2011)**.

Essential oil determination

Twenty grams of the samples were placed in a cap and injected into the soxhlet extractor's internal tube. This equipment was then attached to a flask with a circular bottom that held 200 mL of n-hexane. The arrangement was held to a retort stand and then put on a boiler mantle that was turned on for a 120-minute extraction time at the solvent's boiling point (n-hexane 60^0C). The vapor rises over the tube, is condensed by the condenser, and then falls into the thimble, slowly filling the soxhlet's body. The solvent siphoned over into the flask when it reached the top of the tube, removing the portion of the samples extracted in the thimble. The operation was repeated automatically for a total of 120 minutes before the equipment was removed. Using the same soxhlet extractor, the solvent recovery process was carried out. In the flask, the solvent and oil combination were heated. When the solvent evaporated it was allowed to condense in the thimble chamber after steady heating. Before it was siphoned back into the flask, the solvent was collected. After that, the oil was recovered and its mass was calculated. After extraction and drying in an oven, the mass of the samples was collected in a sample bottle and recorded **(Adama *et al.*, 2011)**.

Preparation of the water extract

For the preparation of water extract, 0.15% (w/v) solution of the samples was used and the solution was heated for 90 minutes, and allowed to cool. The solution was then filtered using Whatman filter paper. The ISO-alpha acids were the determined using the water extract.

Alpha acid and beta acid determination

In this experiment, 0.15 g of the samples were stirred using a flask shaker in 100 ml cold methanol. Following a 20-minute centrifugation of the solution at 2500 rpm, the decanted supernatant was acidified with 0.002 N HCl before being measured with a spectrophotometer at 355, 325, and 275 nm and the α-acid was calculated using (**AOAC, 2000**) method: α-Acid (mg/L) = 73.79 (A325) –51.56 (A355)–19.07 (A275). Beta-acid (mg/L) = 55.57 (A_{355}) –47.59 (A_{325}) +5.10 (A_{275}) Where A is absorbance measured at the stated wavelength

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, *Sacharomyces cervisiae*) 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 121^0 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 20ºC 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 20ºC 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)**.
Er (% Plato) =-460.234+662.649 SG_{ER}-202.414 $(SG_{ER.})^2$

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)**.
Ea (% Plato) =-460.234+662.649 SG_{EA}-202.414 $(SG_{EA})^2$.

Determination of real degree of fermentation

Real degree of fermentation was calculated with the formula,
RDF=100× 2. 0665×A÷2. 0665×A+E_R %)
Where, A=alcohol, % (v/v)
E_R = 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 x 50
Where A = Absorbance at 275nm

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 H_2SO_4. 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 the $Ba(OH)_2$ flask and titrated with HCl the excess barium hydroxide up to the end point **(EBC, 2000)**.

$CO_2 + 2NaOH = Na_2CO_3 + H_2O$
$Na_2CO3 + H_2SO_4 = Na_2SO_4 + CO_2 + H_2O$
$CO_2 + Ba(OH)_2 = BaCO_2 + H_2O$
Excess $Ba(OH)_2 + 2HCl = BaCl_2 + H_2O$

$$\% CO_2 \text{ g/ litre} = \frac{100 (D - E) * 0.0022}{C*A/A+B}$$

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

Determination of total acidity

Ten ml of filtered sample was diluted with 30ml of water and titrated with sodium hydroxide solution using 1ml phenolphthalein; total acidity was recorded as ml of alkali /10 ml of a sample of beer **(Adenuga *et al*., 2010)**.

Determination of vicinal diketones in beer

Vicinal diketones in beer was determined using the spectrophotometric method. In this case, 100 ml of beer sample was measured using measuring cylinder and added to the distillation flask for direct heating until the sample gives 25 ml of the distillate in the measuring cylinder and thoroughly mixed. Ten ml of the distillate and 0.5 ml o-phenylenediamine was pipetted into a dry test tube and kept in a dark place for 20 min. Lastly, 10 ml of water was added into a 50 ml flask with glass and 500 micro liters of OPD was added to prepare the blank reagent **(EBC, 2000)**.

Test for contamination

Contamination test was done to enumerate the possible presence of wort bacteria, wild yeast and lactic acid bacteria were enumerated by spreading 0.1 ml of the sample plates containing wort agar plus actidione, yeast and mold agar plus copper sulfate and universal beer agar with ABP inhibitor respectively. The expression and definition of "no contamination" was defined as "less than or equal to 1 colony forming units per 0.1 ml for wort bacteria and 0 colony forming units per 0.1 ml for both wild yeast and lactic acid bacteria."**(EBC, 2000)**.

Data analysis

The data were analyzed using SPSS version 20.00. Means and standard deviations of the triplicate analysis was calculated using one-way analysis of variance (ANOVA) to determine the significant differences among variables ($p < 0.05$) when the F-test demonstrated significance. The statistically significant difference was defined as $p < 0.05$.

RESULTS

Analysis of bittering agent of *Rhamnus prinoides*

The most valuable bittering components of *R. prinoides* like resins, alpha acids, ISO-alpha- acids, essential oils, and beta-acids were studied**.** For the dosing purpose of beer bitterness, the analyses for determination of content of alpha bitter acids (which are representative for beer bitterness) are important. Some of the components of *R. prinoides* (total resin and bitter acids) are presented in Table 1.

Table 1 Assessment of bittering substances of *R. prinoides*

Samples	Total resin	Hard resin	Soft resin	Alpha acids	Iso-alpha Acids	Essential oil	Beta acids
A	16.02± 0.04^b	9.96±0.02^a	6.24±0.26^a	1.44±0.01^c	1.17±0.05a	3.04±0.05^a	2.18±0.02^a
B	16.14± 0.05^a	9.75±0.26^a	6.39±0.24^a	1.66±0.12^b	1.19±0.02a	3.00±0.01^a	2.14±0.11^a
C	15.96± 0.02^b	9.94±0.04^a	6.02±0.09^a	1.92±0.11^a	1.45±0.47a	3.07±0.06^a	2.07±0.11^a

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

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 eighteen.

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 wort 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 ($p \leq 0.05$) 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 ($p \geq 0.05$) alcohol content compared with other beer types. Beer produced with hop has been shown lower ($p \leq 0.05$) 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^b	8.5±0.02^b
B	6.8±0.01^b	8.4±0.01^b
C	7.4±0.01^a	9.5±0.01^a
D (control)	5.2±0.01^c	6.6±0.01^c

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

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 ($p \geq 0.05$) similar and are different with the other beer types evaluated. Beer type B resulted in relatively ($p \leq 0.05$) 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 shown statistically ($p \leq 0.05$) greater apparent extract (4.80±0.001) in comparison with other beer types. Beer D had been shown statistically ($p \leq 0.05$) less apparent extract (4.20±0.001^c) than the other beer types.

The highest ($p \leq 0.05$) apparent degree of fermentation value (79.72±0.005^a) was observed by beer type D. Beer type C has been shown statistically ($p \leq 0.05$) 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 ($p \leq 0.05$) 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 ($p \leq 0.05$) lower than the rest beer types.

The highest real degree of fermentation value (66.29±0.03) recorded by beer D was significantly ($p \leq 0.05$) 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 value recorded was by beer type C in this study.

Table 4 Value of original extract, apparent extract, real extract and the real degree of fermentation of beer produced by *R. prinoides*

Beer type	Original extract, %p	Apparent extract, mass%	Apparent degree of fermentation, mass%	Real extract, mass%	Real degree of fermentation, mass%
A	20.31±0.01^b	4.80±0.001^a	77.50±0.01^b	8.01±0.01^a	64.93±0.01^b
B	21.60±0.01^a	4.71±0.001^a	77.60±0.01^c	7.90±0.001^b	63.22±0.01^c
C	21.62±0.01^a	4.46±0.002^b	75.91±0.01^d	7.62±0.002^c	62.01±0.005^d
D (control)	18.88±0.01^c	4.20±0.001^c	79.72±0.005^a	5.47±0.01^d	66.29±0.03^a

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

Analysis of Vicinal diketones, bitterness, total acidity, carbon dioxide and pH of beer produced by *Rhamnus prinoides*

Beer type D (0.21±0.01) had been shown statistically ($p \leq 0.05$) lower concentration of vicinal diketone while beer type A (0.25±0.01), beer type B (0.24±.0.01) and beer type C (0.26±.0.01) had shown statistically ($p \geq 0.05$) similar vicinal diketone concentrations.

Based on the result, beer type D (25.0±0.05) have been shown statistically ($p \leq 0.05$) greater bitterness unit than beer type A (2.2±0.15), beer type B (2.4±0.05) and beer type C (2.8±0.1).

Based on the mean value obtained the total acidity of beer type D (0.42±0.01) was shown less ($p \leq 0.05$) total acidity compared with other beer types. All other three beer types have been shown statistically ($p \leq 0.05$) similar values of total acidity.

Based on the mean value of CO_2 obtained in this study, beer type D (0.25±0.01^a) had been statistically ($p \leq 0.05$) greater value of carbon dioxide than the other types of beer.

Beer type A (4.2+0.017) and beer type B (4.3+0.005) have been shown statistically($p \geq 0.05$) similar pH values. The result obtained by beer type D (4.7+0.02) has been significantly ($p \leq 0.05$) 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 g/L	pH
A	0.25±0.01^a	2.2±0.15^b	0.62±0.01^a	0.19±0.01^b	4.2+0.017^c
B	0.24±.0.01^a	2.4±0.05^b	0.61±0.02^a	0.21±0.01^b	4.3+0.005^c
C	0.26±0.01^a	2.8±0.1^b	0.59±0.01^a	0.21±0.101^b	4.4+0.015^b
D (control)	0.21±0.01^b	25.0±0.05^a	0.42±0.01^b	0.25±0.01^a	4.7+0.02^a

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

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

Availability of microorganisms in beer produced by *R. prinoides* was evaluated using a standard microbial culture system. Beer samples were speared on universal beer agar medium and incubated for seven days at 25ºC. 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 variables 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 bittering 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 bittering 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)**. The values of hard resin (9.75-9.96%) of *R. prinoides* was also comparable with *Garcinia cola* (9.69%) which was used as a tropical hop substitute by Adama *et al*., (2011), but less than with that of *H. lupulus*. In the same manner the soft resin (6.02-6.39%) of *R. prinoides* was greater than *Garcinia cola* (2.17%), but less than the commercial *H. lupulus* studied by (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 **(Hieronymus, 2012)**. The evaluated alpha‑acid (1.44-1.92%) and beta‑acid content (2.07-2.18%) of *R. prinoides* was in the range of commercial hop plant 0‑20% **(Hieronymus, 2012)**. It is the alpha-acid that impacts the bitterness in the beer **(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 to be 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, they 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 string 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**).

In this study, beer type A and B apparent extract capacity were similar but, greater than hooped beer. All beer types in this study showed that apparent and real extract were greater than for apparent and real extract of the given beer **(EBC, 2008)**. The original extract of all beer types was within the range of 15.70 and 20.31 %p. A good alcohol-real extract balance is very important for beer taste **(Kunze, 1996)**. Therefore *R. prinoides* can be used as a bittering agent for the production of beer in industrial level.

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 2.50% and 4.42%, respectively **(ES 842, 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 hooped (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 bittering 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 buttery 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 (CO_2) during fermentation is a direct indicator of a good fermentation activity during the brewing process **(Kunze, 1996)**. The CO_2 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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