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Isolation and Characterization of *Saccaromyces cervisiae* Yeasts Isolates from "Tella" for Beer Production

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Authors' contributions

This work was carried out in collaboration between all authors. Author BA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MS conducted the practical activity and author NB managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Saccaromyces cervisiae, isolated from "tella", may serve as potential strains for commercial beer production. The main objective of the current study was to isolate, identify and characterize potential yeast isolates from "tella", which can substitute commercial beer yeast. "Tella" yeast isolates were isolated and identified using biochemical test. Qualities of isolates and physico-chemical characteristics of beer were also determined. The degree of contamination was analyzed. Six yeast isolates were identified and designated as S1, S2, S3, S4, S5, S6, and S7 (commercial yeasts). Yeast viability was determined in wort gravity of 12°p and 14°p and the results were found to be 89 and 83%, respectively,. All isolates showed good fermentative capacity. Isolates S1, S3,

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S4, S5 and S6 grew in 100, 130 and 150 ml/L of absolute ethanol. The flocculation potential of isolate S3 and S4 were 85% and 82%, respectively. Isolates S3 (4.09±.01 v/v %) and S4 (4.36±.04v/v %) showed statistically higher ($p \leq 0.05$) concentration of alcohol than the other isolates. Isolates S1, S5 and S6 showed significantly greater ($p \leq 0.05$) original extract than the control. Isolates S5, S2 and S6 showed statistically greater ($p \leq 0.05$) apparent extract than the other isolates. Isolates had statistically greater ($p \leq 0.05$) apparent degree of fermentation. The physicochemical and other parameters of beers, produced from these isolates, were comparable with those of the commercial *S. cervisiae* isolates. Other threshold substances found in the produced beer were within the standard values of commercial beer. Therefore, *S. cervisiae* isolates from "tella" may be used as a substitute for commercial yeast in beer production.

Keywords: Beer production; characterization; fermentation; Saccharomyces cervisiae isolates; isolation; "tella".

1. INTRODUCTION

Saccharomyces cervisiae is well-known commercially. This organism has long been utilized to ferment the sugars of rice, wheat, barley, and corn to produce alcoholic beverages [1]. It plays significant role in the production of different types of alcoholic beverages like tella, tej and beer.

Beer is the beverage obtained by the alcoholic fermentation of a malted cereal usually barley with or without other starchy materials and to which hops have been added as a bittering and flavoring agent. In brewing, the efficiency of fermentation and quality of the final product are intimately associated with the amount and health of the yeast being used. The physiological condition of pitching yeast may influence the overall organoleptic properties of the final beer product [2].

Yeast quality is usually described in terms of viability or its vitality [3]. Its viability is described as the ability of cells to grow, reproduce and intersect with their immediate environment [4]. Yeast vitality has been variously expressed as a measure of activity, fermentation performance or the capacity to overcome and recover from physiological stress [5].

S. cervisiae with capability of withstanding stressful conditions, high fermentation efficiency, rapid growth, effective sugar use and tolerance of high ethanol concentrations is significant for industrial application. Such yeast isolates can produce high quantity of ethanol with low levels of oxygen and with high capacity of thermo- and osmo-tolerance. *S. cervisiae* with high cell activity in acidic environments is fundamental for industrial applications [6].

In Africa, various kinds of traditional alcoholic beverages such as sorghum beers or opaque beers, have been produced and described [7]. Bilibili is one of these beers that results from the fermentation of sorghum and millet in Chad [7]. "Tella" is one of the Ethiopian traditional beverages, which is prepared from different ingredients [8]. It is, by far, the most commonly consumed alcoholic beverage in Ethiopia. It is assumed that over two million hectoliters of "tella" is brewed annually in households and drinking houses in Addis Ababa alone [9]. There are different types of "tella" depending on type of cereal ingredients. Corn is the most popular, but in some areas barley, millet or sorghum can be used. The way of preparing "tella" differs between ethnic groups and depends on tradition and the economic situation [10]. Even if it may have different names at different localities; fermentation is basically carried out by the activity of *S. cerevisiae.* Here there may be different isolates having basic characteristics to be potential yeast for commercial beer production.

Globally there are a number of brewery factories using specific type of yeast strains, which are capable of producing special flavor and aroma. This study may provide significant information regarding potential yeast isolates that could serve as a starter culture and substitute the commercial yeast strains. Therefore, the objective of this study was to isolate and characterize the indigenous *Saccharomyces cervisiae* of "tella" for beer production.

2. MATERIALS AND METHODS

2.1 Sample Collection

Samples of local beer (*Tella*) were collected from different places in Amhara Region (Mota, Bahir

Dar, Wereta, Debretabor, Gondar town and surrounding villages) of Ethiopia using sterile plastic bottles and transported to the laboratory. The laboratory experiment was conducted with three replications, and maximum care was taken to minimize any variation in the laboratory conditions among treatments.

2.2 Characterization of Yeast Isolates Obtained from "Tella"

Isolation of yeast isolates: Serial dilution (10^{-1} to 10^{-6}) of the samples were prepared using peptone water as diluents and 0.1 ml of each sample was inoculated on yeast peptone dextrose agar containing 50 µg Chloramphenicol/ml using spread plate method [11]. The agar plates were incubated at 30℃ for 48 h.

The yeast isolates were identified according to the method of [12]. Yeast fermentation broth media was used for identification of yeasts based on patterns of specific carbohydrate fermentation [13]. Flocculation test was conducted according to the methods of [14]. To determine ethanol tolerance capacity of isolates**,** yeast isolate was inoculated into 10 ml of liquid YPG supplemented with 100, 130 and 150 ml ethanol per liter and incubated at 30℃ for 72 h [10].

2.3 Propagation of Isolated Yeasts and Fermentation

The yeast inoculums were prepared in wort agar media with chloramphenicol inhibitor (50 µg/ml). A loop full of selected 48 h old white cream colony was inoculated into 50 ml flask containing 5 ml of sterile wort and shaken vigorously using a rotator shaker. The flask was allowed to stand for 24 h. The inoculate wort was transferred into10 ml of sterile wort and this was allowed to stand for another 24 h. This was transferred into another flask containing 20 ml of sterile wort and subsequently to 40 ml and 50 ml sterile cold wort and each was allowed to stand for 24 h to increase the number of yeast cells and incubated at room temperature (25ºC) with constant shaking on a rotary shaker (121 rpm) for 72 h. At the end, 50 ml of fermenting wort was obtained and used as the inoculants for the fermentation process.

2.4 Quality Determination of Isolated Yeast

Yeast cell enumeration: The yeast samples were homogenized and 50 ml of sample was taken and 15 ml of 0.1N sulfuric acid was added. The flocculation phenomena were avoided by acidifying to pH 2.5. One drop of homogenized sample was added into the haemocytometer, using pipette. The prepared slides were allowed to stand for 3-5 min to make the yeast settle and observed on the microscope. The yeast cells, presented in the four corner blocks, were counted.

Yeast cell enumeration calculation was carried out using this formula according to (15):

Where

Total number of cells per ml = A x 4 x 10^4 x B
Total number of cells per 4 blocks = A
Dilution factor = B

Determination of yeast cell viability: In this study, the viability of yeast cells were determined by methylene-blue staining technique, 3 ml of yeast sample was added into 100 ml of beaker containing 50 ml of physiological saline. Two drops of yeast suspension was added into a test tube containing two drops of methylene-blue and the mixture was transferred to the haemocytometer. The total number of yeast cells was counted under 40x bright field objective and the viable cells were viewed as colorless and the dead cells appeared blue in color due to the cell wall of dead cells which absorbed the methylene-blue stain [15].

Yeast cell viability calculation:

Viability = Total number of cell- Number of dead cells/ Total number of cells X 100

2.5 Determination of Physicochemical Parameters of Beer

pH determination: The pH of the beer was determined using pH meter (Mettler Toledo) with combined glass electrode. Fifty ml of sample was poured into 100 ml beaker to rinse the electrode and the rinsing solution was discarded and 50 ml of sample was added into the beaker for test. The measurement was preceded by having the sample in constant movement under the electrode manually by means of magnetic stirrer.

Determination of specific gravity: Specific gravity of the sample was determined using digital density meter (Anton Paar) after 72 h of inoculation of the isolates in the wort sugar. To identify the level of fermentation per day, sample of beer was filtered using density meter at 20℃

daily until the extract arrived at 3 and below the correlation table [16]. At the end of fermentation, specific gravity of decarbonated apparent extract, alcohol and real extract were determined using Refractometer (antonpaar) at 20℃ after distillation [17].

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 was reported as % plato (16) where:

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

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 [18].

$$ER\ (\%\ Plato) = -460.234+662.649\ SG_{ER} - 202.414\ (SG_{ER}.)^2$$

Determination of real degree of fermentation: According to (EBC, 2000), real degree of fermentation was calculated with the formula,

$$RDF=100\times 2.0665\times A \div 2.0665\times A+E_R\ \%$$

Where,

A = alcohol, %(v/v)
E_R = real extract, Plato

Determination of Alcohol content: Alcohol content was determined by using distillation by direct heating and determining the alcohol % (w/w) from the distillate specific gravity, the alcohol % (v/v) was determined from the specific gravity of the filtered beer and alcoholic % (w/w) [18].

Determination of carbon dioxide in beer: Carbon dioxide of the beer was determined by the carbon dioxide bloom method. Determination of vicinal diketone (VDK) in beer was conducted using [19] method.

Determination of bitterness in beer: The bitter compound was extracted with Iso octane from acidified beer and the absorbance was measured at 275 nm using quartz cuvette [14]. Ten ml of degassed beer was pippeted into 35 ml centrifuge tube or a 50 ml conical flask, and then 0.5 ml of HCl was added into degassed beer. The iso-octane solution was placed into 2 or 3 glass balls in the centrifuge tube. The cap was screw with polythene, and inserted on to the centrifuge tube. The centrifuge tubes were shaken for 15 min at 20+/10℃ using rotary shaker set at 130 rpm, the sample was shaken until the maximum extraction was achieved [13].

2.6 Test for Contamination

Wort bacteria, including lactic acid bacteria and also wild yeasts, were enumerated by spreading 0.1 ml of the sample plates containing wort agar plus actidione, yeast and mould agar plus copper sulfate and universal beer agar with ABP inhibitor respectively [20]. 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 yeasts and lactic acid bacteria were defined as no contamination [21].

2.7 Data Analysis

All data were analyzed using SPSS version 16.0. Means and standard deviations of the triplicates analysis was calculated using analysis of variance (ANOVA) to determine the significance differences between the means followed by Duncan's Multiple range test ($p < 0.05$) when the ANOVA test demonstrated significance. The statistically significant difference was defined as $P < 0.05$.

3. RESULTS

3.1 Morphological Characteristics of Yeast Isolates from "Tella"

A total of six yeast isolates were selected and identified based on morphological characteristics such as colony morphology (colony shape, color and surface appearance), cellular vegetative morphology (cell shape and arrangement) as described in Table 1. The isolates were designated as S1, S2, S3, S4, S5, S6 and S7 (S7, standard or commercial yeast strain). The size and shape of cells and the patterns of vegetative propagation are characteristic of individual yeast species and may be used as aids for identification.

3.2 Utilization of Carbon by Yeast Isolates from "Tella"

Carbon utilization capacities of each isolates are shown on Table 2. All isolates metabolized glucose, galactose, fructose, and sorbitol but not

Table 1. Morphological characteristics of yeast isolates, isolated from "tella"

Yeast isolates	Colony shape and color	Colony surface appearance	Vegetative morphology cell shape arrangement	Budding
S1	Creamy and spherical	Smooth and flat	Spherical cell	Single
S2	White to creamy spherical	Smooth and flat	Spherical elongated cell, Oval cells	Multi polar
S3	Creamy and spherical	Smooth and flat	Spherical elongated cell	Single
S4	Creamy and spherical White	Smooth shiny and flat	Spherical elongated cell	Multi polar
S5	Creamy and spherical	Smooth and flat	Spherical elongated cell	Multi polar
S6	Creamy and spherical	Smooth and flat	Spherical elongated cell	Single
S7	Creamy and spherical	Smooth and flat	Spherical elongated cell	Single

Table 2. Metabolism of carbohydrate by *S. cervisiae* species, isolated from "tella"

Isolates	S1	S2	S3	S4	S5	S6	S7
Glucose	+	+	+++	+++	++	+++	+++
Galactose	++	++	++	++	++	++	++
Fructose	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-
Sucrose	+	++	++	++	-	+	+++
Mannitol	-	-	-	-	-	-	-

+ = Positive response; - = Negative response

lactose and mannitol. Isolate S5 did not metabolize sucrose at all. The extent of each carbon fermentation potential of isolates were variable. Sucrose fermentation performance of isolates S2, S3, S4 and S7 was better than that of the other yeast isolates. Fructose and sorbitol fermentation performance of all the isolates were moderate compared with other carbon sources.

3.3 Quality Determination of Yeast Isolates

3.3.1 Analysis of viability of yeast isolates in propagation of 12 and 14 ^{o}p wort sugar concentration, yeast count and specific gravity of fermentation test

The potential of viability of each yeast isolates in propagation of 12 and 14 ^{o}p wort sugar concentrations is presented on Table 3. High gravity wort used for propagation had a negative effect on yeast viability during fermentation. The maximum Yeast viability in wort gravity of 12°p and 14°p were showed by S7 strains which was 96.6 and 94.33, respectively, at the end of fermentation. The yeast viability capacity of all isolates in high gravity fermentation reduced with increased wort gravity during propagation. The viability of isolates S7 at 12°P and 14°p were 96.66±0.58 and 94.33±0.57%, respectively. Therefore, the viability of isolate S7 was significantly ($P \leq 0.05$) greater than the rest.

Yeast count and specific gravity of fermentation by yeast isolates from "tella" is shown in Table 3. The degree of extract decreased with fermentation time. According to this finding, yeast isolates designated as S3 and S4 had good fermentative capability in comparison with other isolates. Generally, yeast isolates from "tella" had good fermentative capacity in comparison with commercial *S. cervisiae* under this study.

3.3.2 Ethanol tolerance of *S. cervisiae* isolates from "tella"

Ethanol tolerance of yeast isolates from "tella" is shown in Table 4. Growth of yeast isolates was retarded with increasing absolute ethanol concentrations. The isolates S1, S2, S3, S4, S5, S6 and S7 showed intensive growth at 80 ml/L of absolute ethanol while growth was moderate in 100 ml/L absolute alcohol. Scanty growth was observed in 130 and 150 ml/L concentration of absolute ethanol by isolates S1, S3, S4, S5 and S6. S2 and S7 isolates failed to grown in 150 ml/L absolute ethanol.

3.3.3 Flocculation capacity of yeast isolates from "tella"

In this study, the flocculation potential of isolates S3 and S4 were 85% and 82%, respectively. However, the flocculation capacity (97%) of commercial *S. cervisiae* (control) (S7) was better than all the yeast isolates obtained from "tella". According to the current study, the flocculation potential of yeast isolates of S2 (50%) and S5 (65%) were lower than the rest of the yeast isolates.

3.4 Physico-chemical Characteristics of Beer Produced from Different *S. cervisiae* Isolates

Alcohol content of beer produced by different *S. cervisiae* isolates is presented in Table 5. Isolates S3 (4.08±.0.01 v/v %) and S4 (4.08±.0.01%) were statistically ($P \le 0.05$) greater in alcohol concentration than isolates S1 (3.55±.0.5%), S2 (3.55±.0.05%) and S5 (3.75±.0.08%). The lowest alcohol concentration was observed (3.21±0.26%) by isolate S6.

Apparent extract and real extract of beer produced by different *S. cervisiae* is shown in Table 5. Isolates S1, S5 and S6 showed statistically ($P \le 0.05$) significantly greater original extract than other isolates, including control strains S7). Beer, produced by isolates S6 (8.5733 °P) and S5 (8.5033 °P) showed statistically ($P \le 0.05$) greater real extract than that of the other isolates, which include strain S7 (the commercial strains).

Isolates S5 and S2 showed statistically ($P \le 0.05$) greater apparent extract (6.7, 6.74 °P and 6.78 ^{0}P, respectively) than all the isolates. Isolates S3 and S4 showed statistically ($P \le 0.05$) less apparent extract (4.80 °P and 4.90 °P, respectively) than isolates S1, S2, S5 and S6.

The highest real degree of fermentation value was 62.14 and 61.22% by isolates S6 and S5. The lowest value observed (46.30%) was by isolates S2. Isolate S6 showed statistically ($P \le 0.05$) greater real degree of fermentation (62.14%) than all the isolates.

The highest apparent degree of fermentation was observed (75.71%) in the commercial yeast and the lowest apparent degree of fermentation observed (35.71%) was by isolate S5. Isolate S6 had statistically ($P \le 0.05$) greater apparent degree of fermentation (62.20%) than isolates S1, S2, S3, S4 and S5 (Table 5).

3.4.1 Analysis of VDK, bitterness, pH and carbon dioxide of beer produced by different yeast isolates

Isolates S6 and S4 showed statistically ($P \le 0.05$) greater concentration of vicinal diketone (0.21mg/l and 0.21mg/l, respectively) than all other isolates (Table 6). Isolate S7, the commercial strain, showed statistically less ($P \le 0.05$) vicinal dietone concentration (0.13mg/l) than all other isolates (S1, S3, S4, S5 and S6). There was no statistically significant ($P \ge 0.05$) difference between isolates S1 and S3.

Based on the result, isolate S1 showed statistically greater ($P \le 0.05$) amount of bitterness (26.94) than all the isolates. Strain S7 showed statistically less ($P \le 0.05$) amount of bitterness (20.28) than all isolates. Isolates S4 showed statistically lower ($P \le 0.05$) amount of bitterness than all the isolates. There was no statistically significant ($P \ge 0.05$) difference between isolates S3 and S5 as far as bitterness is concerned (Table 6).

Based on the result, the commercial isolates showed statistically ($P \le 0.05$) greater pH value (4.52) than all the other isolates. Isolates S2 and S1 showed statistically less ($P \le 0.05$) pH value than all other isolates. Isolate 4 had statistically ($P \le 0.05$) greater pH value (4.35) than isolates S1, S2, S3, S5 and S6 (Table 6).

Table 3. Analysis of viability of *S. cervisiae* species in propagation of 12 °p and 14 °p wort sugar concentrations, yeast count and specific gravity of fermentation test

Isolates	Viability in 12°p (%)	Viability in 14°p (%)
S1	85.67± 0.58^{a}	79.00±1 ^{a}
S2	87.00±1 ^{ab}	81.33±.58^{a}
S3	93.00±1 ^{cb}	90.33±.58^{d}
S4	91.00±1 ^{cb}	85.33±8.9^{c}
S5	83.00±1 ^{a}	76.00±4.3^{a}
S6	87.00±1 ^{ab}	80.66±.57^{a}
S7	96.66±.58 ^{d}	94.33±.57^{d}

Isolates	Sg & YC of day 4		Sg & YC of day 8		Sg & YC of day 12		Sg YC of day 16		Sg & YC of day 18	
	Sg (°P)	CFU/ml	Sg(°P)	CFU/ml	Sg(°P)	CFU/ml	Sg (°P)	CFU/ml	Sg (°P)	CFU/ml
S1	11.872	0.066	7.057	0.213	6.637	0.220	4.312	0.173	3.721	0.095
S2	11.411	0.048	7.348	0.208	5.864	0.231	4.001	0.189	3.213	0.101
S3	9.352	0.179	4.814	0.261	3.008	0.141	-	-	-	-
S4	8.281	0.191	4.977	0.281	3.470	0.153	-	-	-	-
S5	11.315	0.071	6.943	0.201	4.945	0.238	3.053	0.165	2.713	0.121
S6	9.674	0.057	7.129	0.199	4.922	0.229	2.944	0.152	-	-
S7	4.931	0.372	2.114	0.081	-	-	-	-	-	-

-= No fermentation, Sg= Specific gravity, Yc = Yeast count

Specific values in the above Table are means of triplicate determinations. Values with different superscripts within the column are significantly different at (P< 0.05)

Table 4. Ethanol tolerance of yeast isolates from "tella"

Isolates	70 ml/L	80 ml/L	100 ml/L	130 ml/L	150 ml/L	160 ml/L
S1	+++	+++	++	+	+	-
S2	+++	+++	++	+	-	-
S3	+++	+++	++	+	+	-
S4	+++	+++	++	+	+	-
S5	+++	+++	++	+	+	-
S6	+++	+++	++	+	+	-
S7	+++	+++	++	+	-	-

+++ (Intensive growth), ++ (Moderate growth), + (Little growth), - (No growth)

Table 5. Analysis of alcohol content of beer produced by different *S. cervisiae* isolates

Isolate	Ac (w/w %)	Ac (v/v %)
S1	3.22±0.3^{b}	3.55±0.5^{a}
S2	2.16±0^{a}	3.55±0.05^{ab}
S3	3.77±0.05^{c}	4.08±0.01^{cd}
S4	3.91±0.01^{c}	4.35±0.04^{d}
S5	2.54±0.6^{a*}	3.75±0.08^{c}
S6	2.15±0.01^{a*}	3.21±0.26^{a}
S7	5.04±0.01^{d}	6.53±0.01^{e}

Isolate	Original extract (Ᵽ) (%)	Apparent extract (Ᵽ) (%)	Real extract (Ᵽ) (%)	Apparent degree of fermentation Mean (%)	Real degree of fermentation Mean (%)
S1	14.13± 0.2^{b}	6.21±.2^{c}	7.48±.1^{c}	52.70±.1^{c}	50.52±.02^{b}
S2	13.75±.0.1^{a}	6.74±.05^{d}	8.11±.04^{d}	40.33±.5^{b}	46.30±.01^{a}
S3	13.60±0.2^{a}	4.80±.03^{b}	6.55±.04^{b}	54.23±.03^{d}	56.45±.01^{c}
S4	13.33± 0.1^{a}	4.90±.1^{b}	6.74±.05^{b}	54.67±.02^{e}	59.80±.01^{e}
S5	14.08 ± 0.3^{b}	6.70±.1^{d}	8.50±.1^{e}	35.71±.01^{a}	61.22±.02^{f}
S6	14.29±0.2^{b}	6.78±.03^{d}	8.57±.1^{e}	62.20±.08^{f}	62.14±.02^{g}
S7	13.01±0.1^{a}	3.40±.02^{a}	4.80±.3^{a}	75.71±.3^{g}	58.44±.2^{c}

Table 6. Analysis of VDK, bitterness, pH and Carbon dioxide of beer produced by different yeast isolates

Isolate	Vicinal di ketone (mg/l)	Bitterness (IBU)	pH	Carbon dioxide (%)
S1	0.15 ± 0.01^{c}	26.94 ± 0.06^{f}	3.68 ± 0.07^{a}	0.38 ± 0.01^{a}
S2	0.13 ± 0.01^{b}	21.66 ± 0.01^{c}	3.67 ± 0.03^{a}	0.44 ± 0.01^{b}
S3	0.16 ± 0.01^{c}	23.67 ± 0.01^{e}	4.21 ± 0.01^{e}	0.44 ± 0.02^{b}
S4	0.21 ± 0.01^{e}	20.83 ± 0.02^{b}	4.35 ± 0.01^{d}	0.46 ± 0.01^{c}
S5	0.17 ± 0.01^{d}	23.68 ± 0.03^{e}	4.02 ± 0.02^{c}	0.38 ± 0.01^{a}
S6	0.21 ± 0.01^{e}	22.46 ± 0.02^{d}	3.92 ± 0.02^{b}	0.45 ± 0.01^{b}
S7	0.12 ± 0.01^{a}	20.28 ± 0.04^{a}	4.52 ± 0.02^{f}	0.51 ± 0.01^{d}

Specific values in the Table 5 are means of triplicate determinations. These values with different superscripts within the column were significantly different at (P< 0.05)

Based on the present study, the mean value of CO_2 for isolates S3, S4 and S6 showed statistically ($p \leq 0.05$) greater concentration of carbon dioxide (0.44, 0.46 and 0.45, respectively) than all the remaining isolates. Strain S7, the commercial strain showed statistically ($P \leq 0.05$) greater concentration of carbon dioxide (0.51%). Isolates S1 and S5 showed less concentration of carbon dioxide (0.38%) than all isolates that showed statistically significant value ($p\leq0.05$) as described in Table 6 above.

3.5 Detection of Microbial Contaminant in Beer Produced by Yeast Isolates

The microbiological analyses indicated the absence of mold, wort, bacteria and lactic acid bacteria in these products hence they are safe to drink. The sterilized wort, pure yeast and the sterilized and cooled vessel were free from any contaminants.

4. DISCUSSION

The wort gravity used in the propagation process had profound effect on yeast cell volume. High wort gravity increases the yeast stress. As a result, it affects the cell size and changes the cell volume due to its osmotic effect. All these effects are attributed to high wort gravity that imposes stress on yeast isolates [22].

In this study, high gravity of wort used for propagation of yeast isolates showed negative effect on yeast viability during the fermentation process. All isolated and identified *S. cervisiae* showed the same phenomena. Yeast propagation in wort gravity of 12°p was found to be suitable for all *S. cervisiae* isolates. Therefore, yeast cells were able to survive with certain limits of wort concentration. Generally, the isolated and identified yeast isolates had a good viability capacity at 14°P.

One of the most important technologically valuable characteristics of yeast is its ability to ferment the extract rapidly. According to the present finding, the various yeast isolates behaved in different ways as far as their fermentative capability is concerned. Isolates identification can be based on assessment of performance in laboratory wort fermentations. Generally yeast isolates from “tella” showed good fermentative capability as compared to the commercial *S. cervisiae* strains, which are used as controls for this study. Local beer (Tella) can be the best yeast isolates source and may use in the future for commercial beer brewing process. Moreover, the finding of the present study provides a promising source of noble beer yeast isolates with quality aroma and color.

Ethanol is the main extracellular metabolite of *S. cervisiae* in anaerobic fermentation. It exerts a very notable influence on growth velocity and fermentation rate of yeasts. In this study, ethanol tolerance (150ml/L) of five isolated yeast were greater than commercial (control) yeast strains. It is well documented fact in the literature that different *S. cervisiae* isolates have different capacity for resisting concentration of alcohol. *S. cervisiae* isolates of this study were in line with the report of [23] in respect to capacity of alcoholic resistance. One of the best qualities of yeast isolates for beer production is the capability of tolerance of high alcohol concentration. It is well known that the cell volume of yeast is significantly altered with increasing ethanol concentration. This is because the ethanol stress can reduce cell volume [24].

High flocculation capability of beer yeast strains is another parameter for selection of yeast for commercial purpose. Flocculation occurs because of interactions between surface proteins

on one cell and carbohydrate receptors on another cell [14]. Determination of the flocculation behavior of yeast isolates and populations is significant to get appropriate yeast isolates for beer production [25]. In this study, onset of flocculation was observed in laboratory cultures that just entered the stationary phase of growth. Similarly, in brewing, flocculation occurs towards the end of primary fermentation [26]. In the present finding, flocculation capacity of the isolates was a little bit lower than commercial yeast isolates. The flocculation is an important characteristic that allows an easy separation of the final product at the end of the fermentation without additional filtration/centrifugation steps and also allows the utilization of immobilized yeasts on fermentation processes [27].

The analysis of beer for alcohol content is an important part of brewing to maintain quality assurance programs and legal reporting purposes. The alcohol content of beer produced by isolates S3 (4.09%) and S4 (4.36%) were almost comparable with commercial yeast strains in this study and lager beer (4.7-5.0%). Most yeast isolates of this study w/w and v/v values of ethyl alcohol were within the standard value of string beer. Ethanol is by far the main extracellular metabolite of S*accharomyces* yeasts in the anaerobic fermentation that exerts a very notable influence on growth velocity and fermentation rate of yeasts [28]. Therefore, yeast isolates of this finding provided a very good alcohol tolerance capacity to solve influence on growth velocity and fermentation rate of yeasts.

In this finding, yeast isolates S6 and S5 apparent and real extract capacity were comparable with commercial yeast isolates (control). All yeast isolates investigated in this study were in line with desired extract for apparent and real extract of given beer [17]. The original extract capacity of all yeast isolates were within the range of 13.33 and 14.29°P. This value is within the standard value of extra strong beer (12.51-14.50°P) (ES833, 2012). Therefore, all isolates can be used for production of industrial extra strong beer.

Real degree of fermentation refers to the percentage of reductions in wort's specific gravity caused by the transformation of the sugars into alcohol and CO_2 and yeast biomass. The apparent degree of fermentation of all yeast isolates were less than that of the commercial (control) yeast while real degree of fermentation capacity of most yeast isolates tested in this investigation were in agreement with commercial yeast isolates . The minimum standard values of apparent and real extract of extra strong beer are 2.50% and 4.42%, respectively (ES 842, 2012). The minimum apparent and real extract of the beer produced from isolated yeast were 4.80% and 6.55%, respectively. The control (commercial strain) apparent and real extract values were 3.40% and 4.80%. Therefore, the apparent and real extract values of yeast isolates in this study were by far greater than the standard minimum values for extra strong beer. All isolated yeast organisms in the present study may be used for production of commercial extra strong beer.

Vicinal diketones make the beer to have a sweet, non-specific taste. If its concentration is over the limit value, it causes the beer to have buttery aroma [29]. Vicinal diketone concentration of beer produced from all isolated and tested yeast isolates were greater than the beer produced by commercial yeast isolates. Generally, beers produced by some yeast isolates of this study have a little bit greater vicinal diketones values than the standard value (0.15 mg/L) of (ES843, 2012). Formation and reduction of vicinal diketone is affected by yeast isolates, type of fermentation temperature, wort composition, aeration and amount of dissolved oxygen, pH, bacterial infection and pressure [29-31]. If these are true, further study and isolates improvement techniques are important to reduce the amount of vicinal diketones of beer in this investigation.

Beer bitterness is primarily caused by the hop but in addition poly-phenol bitterness can also attribute to the amount of protein bitterness and yeast bitterness [32]. Apart from hop, bitterness of the beer is also affected by type and amount of malt used in production process. In order to maintain consistency in quality, bitterness needs to be strongly monitored and controlled. Different types of beers tend to have different IBU values that range from 0 to 100 IBU [33]. In this investigation, the substrate (malt, hop and water) provide for each yeast isolate (from all yeast isolates) were equal. The only difference was the yeast isolates type. In the present study, the beer bitterness difference may be caused by yeast isolates type. Whenever bitterness is compared among the different isolates, S1 provided greater amount of bitterness. On the other hand, the beer produced by commercial strain had less bitterness. The degree of bitterness of beer in this investigation was within the range of 21.66 to

26.94 IBU. All beers produced were within the range stated by [34].

The pH value of beer produced by different yeast isolates were within the range of 3.67±0.01 and 4.35± 0.01. The pH values of beers produced from different isolates of the study were within the standard value (3.6-4.8) of (ES830, 2012) and Indian standard beer specification value (3.8 – 4.5) (BIS, 2001). The pH in such a way can reduce the risk of contamination and secondary fermentation which are associated with traditional honey wine production [34].

Normally, the amount of released CO_2 during fermentation is a direct indicator of fermentation activity of yeast. The CO_2 content of beer is one of its most important quality criteria. Beers with a good amount of foam have a CO_2 content of 0.45 to 0.50% about 15% of the CO_2 content produced remain dissolved in beer. The largest part escapes during fermentation and can be available for CO_2 recovery for different purposes [28]. In this finding, beer produced by isolates S3, S4 and S6 showed higher concentration of CO_2 than the rest isolated yeasts. In general, except yeast isolates S1 and S5, the CO_2 content of the beers produced by all isolates of this finding were in line with the standard value of commercial beer (ES831, 2012). However, the beer produced by commercial yeast strains provided greater concentration of CO_2 than all isolated yeasts. High concentration of CO_2 above and over the limit in the beer affects the growth and multiplication of yeast due to the formation of carbonic acid (HCO_3^- ions) and thereby reduced the pH of the yeast medium [28].

Determination of microbial profile of beer produced from isolated yeasts is significant to evaluate the quality of beer. Beer contaminants are well known to cause beer infection and as a result deteriorate the quality of beer. Usually wort bacteria appear, if there is poor hygiene in the fermenting vessels and yeasts. In this study, sterilized wort, pure yeast and the sterilized and cooled vessel were free from any contaminant. Mostly, lactic acid bacteria are known to reduce the pH value of beer below standard. The lactic acid bacteria (LAB) are rod-shaped bacilli or cocci characterized by an increased tolerance to a lower pH range. This feature may help LAB to outcompete other bacteria in a natural fermentation, as they can withstand the increased acidity from organic acid production (e.g., lactic acid). However, they may increase the acidity of the product, beer [34].

5. CONCLUSION

"Tella" can serve as best source of different *S. cervisiae* isolates for production of different types of beer. The beers produced from different isolates had properties which were in line with that of commercial beer produced by commercial *S. cervisiae* isolates. Specially, flocculation capacity and ethanol tolerance potential of isolated *S. cervisiae* in the present study were better than the control *S. cervisiae* strain. Other threshold substances found in the beer produced were within the standard values of commercial beer. The isolates of *S. cervisiae* obtained in this study can be used for commercial beer production. If utilized in large scale the *S. cervisiae* obtained can replace commercially produced yeast strains used for beer production and minimize the resource being spent for importing yeast strains. But prior to use for industrial activity, pilot study should be conducted. More research should be conducted on "tella" produced from different part of Ethiopia to isolate and identify more potential *S. cervisiae* isolates that can be used as yeast source for industrial beer production. Molecular characterization and further analysis of yeast isolates is warranted to determine the quality and variation among isolates and determine them at strain level.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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