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Characterization of wild yeasts isolated from cereal sourdoughs and their potential for leavening wheat dough

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Abstract

Fermentation of cereal-made dough was the primary Ethiopian food for many years. Sourdough is believed to have many beneficial fermenting micro-floras depending on the source of dough and environmental conditions. The study aimed to isolate and characterize yeasts from fermenting cereal dough and evaluate their leavening potential for wheat dough. Morphological and biochemical characterizations such as sugar fermentation, glucose tolerance test, growth at different pH and temperatures, and agitation effect were employed to select potent yeasts. Accordingly, 16 potential yeast isolates were selected and subjected to further analysis. Finally, the yeasts were tested and compared for their leavening activity on the wheat dough at 37 °C and room temperature. Results revealed marvelous CO_2 producer mesophile yeasts that degrade various sugars and tolerate up to 60% glucose concentration. The overall characterization assigned all the yeast isolates to the genus *Saccharomyces* and the *Candida humilis.* ANOVA showed that leavening activity by the yeast isolates was statistically higher at 37 °C compared to room temperature. The highest dough volume (6.2 mL) was recorded from Wh17, Wh5, and Ba12 isolates. Sor9 isolates were among the yeast that raised a minimum dough volume (0.7 mL). In contrast to commercial yeasts, the present study detected exceptional yeasts that matured within 24 h. Therefore, the discovered potential yeasts can be used for leavening dough in a short fermentation time and add aroma to baked products, making them a candidate in the baking industry. It could also be cost-effective as it could reduce media consumption and electricity utilization.

Keywords Fermentation · Sourdough · Sugars · Wheat dough · Cereal · Yeast isolates · Fermentation time

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1 Introduction

Fermentation of food using natural microbiota has been used for centuries to preserve food and enhance its flavor [1]. One of the first techniques of grain fermentation was performed in ancient Egypt around 4000 B.C. using yeasts and lactic acid bacteria [2]. Although the yeast's primary function is to convert sugar to CO_2 and leaven the dough through alcoholic fermentation, it also has a significant effect on the flavor and crumb structure of baked goods [3]. The use of sourdough has already become more methodical to ensure the continuation of fermentation, using a part of the ferment to create and spread microbial cultures for later use.

Sourdough is an acidic, sharp-tasting flour and water mixture used to make bread [4], and *Injera* (Ethiopian bread made from the fermentation of grain flour). It has also been used as a leavening agent in the preparation of various baked products throughout the history of baking [5]. By slowing down the staling process and reducing mold growth, sourdough improves the volume, texture, flavor, nutritional value, and sensory aspects and extends the shelf-life of baked goods [6, 7]. In Japanese bread making, natural yeast is used instead of commercial dry yeast [8]; its composition and activity determine the influence of sourdough on bread quality [9]. Starter cultures are still rarely used directly in bread making. Instead, bakeries propagate sustained sourdoughs from batch to batch using a portion of the previous batch as a starter culture. However, this approach does not guarantee consistent fermentation microbiota, which in practice does not affect product quality during baking [10]. As a result, scientific information is essential to increase public awareness of sourdough. For years, scientists have been isolating and discovering different yeasts that are excellent for winemaking, beer brewing, and baking. Yeast grows on low-cost substrates, including wastes, and their large size and flocculating properties allow for a more cost-effective harvest. As a result, it is a microbiota that is well suited for experimentation. The raw materials used as substrates for industrial yeast biomass production are usually agricultural wastes (starch, fruit, and vegetable wastes), forestry wastes (wood, straw), food wastes, industrial wastes (molasses, distillery water), etc. [11]. It is expected that different yeasts can occur in different grain-made sourdoughs.

Ethiopian sourdough is traditional fermented grain dough prepared from various ingredients such as barley, wheat, sorghum, *Teff*, and maize [12]. Previous researches have demonstrated that these grains have the correct proximate composition, including carbohydrates, proteins, fats, fiber, and minerals including Fe, Mn, Mg, P, K, Na, Zn, and ash, as well as essential vitamins necessary for cell growth [13–22]. In Ethiopia, the traditional fermentation of grain flour is carried out using naturally occurring microbes. To keep the fermentation process going, they use some of the previous sourdoughs and mix it with new flour to ferment, assuming that they are using a fermenter/microbes. Among the microbiota, yeast is likely to be the major player in the fermentation of grain dough [23]. Wild fermenting yeasts are continually being hunted for further commercial use in the baking industry. Sourdough wastes are recommended as promising substrates for the growth and proliferation of yeasts in industrial baking applications [24]. Ethiopia's population of over 100 million has a significant demand for baker's yeast to produce enough bread. Most breadbakers in Ethiopia use starter culture (baking powder) imported from foreign countries [25, 26]. Characterizations of the microflora present in local foods and optimization of their use as starter cultures have not yet been exploited, particularly in bread making. Thus, this study was carried out to isolate, identify, and characterize yeast isolated from Tigray traditional grain sourdoughs for prospective use in dough leavening in the baking industry and for home consumption in communities where *Injera* is the main meal. Identifying effective yeast strains allows us to optimize the fermentation of grain dough and produce homogeneous food products with high nutritional value. This enables valuable fermented products to be produced quickly and economically.

2 Materials and methods

2.1 Sample collection and substrate characterization

Maize, barley, wheat, finger millet, *Teff*, and sorghum were selected for yeast isolation and samples were collected from different districts of Tigray National Regional State (Sheraro, Shire, Axum, Adigrat, Qola Temben, Mekelle, and Korem), Ethiopia, and transported to the biotechnology laboratory of Mekelle University, Ethiopia, for further analysis. The samples were stored at room temperature for a week until analyzed. An amount of 15 kg of each grain, including maize, barley, wheat, finger millet, *Teff*, sorghum, and beans were randomly purchased from local markets in the designated districts. These are among the widespread crops in Tigray, Ethiopia, whose fermentation products form the staple food.

2.2 Sample preparation

Samples of maize, barley, wheat, finger millet, *Teff*, sorghum, and were washed with sterile distilled water and dried in the sun for one day. They were then ground into fine powder in a mill (Model BT-450, India), sieved, and packed in sterile plastic bags. Fermentation was carried out in the typical household manner by initially mixing 1 kg of grain flour from each sample with 2 L of sterile distilled water in a fermentation plastic jar in a ratio of 1:2 (1 kg flour per sample to 2 L of distilled water). The mixture was then stirred thoroughly until a uniform suspension was formed. The batter was placed in a restricted space at room temperature (24.5 °C) for 96 h to allow fermentation [27].

2.3 Isolation of yeast

A fermented grain mixture based on sorghum (Sor), finger millet (Fm), barley (Ba), *Teff* (Te), maize (Maz), wheat (Wh), and beans (Be) were used as a source for yeast isolation. To isolate yeast, 1 mL of each sample was transferred to 9 mL of sterile saline water and mixed thoroughly. From the prepared ten-fold serial dilution (10^{-1}–10^{-6}), 0.1 mL of diluted samples were aliquoted and spread on potato dextrose agar (PDA) plates (HIMEDIA, India) containing g/L (Potatoes, infusion from, 200; Dextrose, 20; Agar 15). An appropriate amount (1 µg/mL) of chloramphenicol (Sigma-Aldrich, USA) was added to the agar to inhibit bacterial growth. Colonies were selected based on their shape, size, and color from samples incubated at 30 °C for 48 h. Repeated sub-culturing on freshly prepared potato dextrose agar (PDA) (HIMEDIA, India) was employed to get a pure colony. Pure isolates were inoculated on PDA slants (HIMEDIA, India) and stored in the refrigerator at 4 °C for subsequent investigation [28]. The active dry baker's yeast (Saf instant) from DSM Bakery Ingredients, Holland commercial yeast (Cy), was utilized as a control.

2.4 Staining and biochemical characterization of yeast isolates

Yeast isolates were stained with methylene blue and inspected under a microscope using a standard procedure [29]. The carbon dioxide test was performed in yeast extract peptone D-glucose (YEPD) broth (HIMEDIA, India) containing g/L (yeast extract, 5; peptone, 5; D-glucose, 10) according to the standard methodology reported by [30]. Furthermore, the carbohydrate fermentation test of prospective yeast isolates was analyzed and provided as follows;

2.4.1 Carbohydrate fermentation test

A carbohydrate fermentation test was performed following the previously described standard procedure [31] to evaluate the potential of yeasts to utilize different carbon sources. Accordingly, 10% solutions of glucose, fructose, sucrose, maltose, galactose, xylose, sorbitol, and lactose (Nice Chemicals, India; Sigma-Aldrich, USA) were prepared and filter-sterilized via a Millipore (Model WP6122050, USA). After sterilization of the YEPD broth (HIMEDIA, India) with g/L (yeast extract, 10; peptone, 10 g; Bromocresol purple, 2% (w/v)) bromocresol purple in an autoclave (Model AM B240, United Kingdom) at 121 °C for 15 min. Then 0.5 mL of each sugar solution was added to 5 mL of broth. The carbohydrate fermentation test was carried out by inoculating loop-full yeast colonies into tubes containing 5 mL of broth enriched with various sugars (10%, w/v) and incubating at 30 °C for 48 h. Finally, the result was evaluated qualitatively/visually by analyzing the color changes in the solution after three days of incubation.

2.5 Examining factors affecting yeast growth

The yeasts' responses to several fermentation parameters such as temperature, pH, glucose, NaCl, and agitation were measured. Accordingly, the ability of yeasts' enzymatic activity to function at different temperatures, the pH range in which yeast grows best, the capacity of the yeast to withstand osmotic stress at different sugar concentrations, the ability of the yeast to grow in the presence of a high salt concentration, and the effect of agitation on the yeast growth were all evaluated. The detailed experimental protocols used to test the influence of these factors on yeast growth are provided in the following sections;

2.5.1 The effect of different temperatures on yeast growth

To evaluate the capability of yeast isolates to grow at different temperatures, each isolate was inoculated in 50 mL of YEPD broth (HIMEDIA, India) containing g/L (yeast extract, 5; peptone, 5; D-glucose, 10) with the pH adjusted to 5 using 1N HCl (Sigma-Aldrich, USA) and 1N NaOH (Nice Chemicals, India) and sterilized in an autoclave (Model AM B240, United Kingdom) at 121 °C for 15 min. Tubes containing this medium were inoculated with the same number of actively growing yeast cells (approximately 3.6×10^6 CFU/mL) and incubated at four different temperatures (27 °C, 32 °C, 37 °C, and 42 °C). The optical density at 550 nm was determined using a spectrophotometer (Model 6405 UV/Vis, JENWAY, United Kingdom) at intervals of 24 h, 48 h, and 72 h as a measure of growth [32].

2.5.2 Growth of yeasts at different pH values

YEPD liquid medium (HIMEDIA, India) with g/L (yeast extract, 5; peptone, 5; D-glucose, 10) was prepared in a separate flask, and the pH was adjusted to 4, 5, and 6 with 1N HCl (Sigma-Aldrich, USA) and 1N NaOH (Nice Chemicals, India). Selected yeast isolates were then subjected to the specified pH ranges to examine their tolerance potential by inoculating about 3.6×10^6 CFU/mL yeast cells in 50 mL YEPD broth and incubating at 30 °C. Growth was determined after 48 h by measuring the optical densities at 550 nm using a spectrophotometer (Model 6405 UV/Vis, JENWAY, United Kingdom) [33].

2.5.3 Glucose tolerance test

For the glucose tolerance test of yeast isolates, an appropriate amount of glucose (Nice Chemicals, India) was prepared and filter-sterilized using Millipore (Model WP6122050, USA). For this purpose, yeast-malt agar (HIMEDIA, India) containing g/L (Malt extract, 3; Yeast extract, 3; Peptone, 5; Dextrose, 10; Agar, 20) was used as the basal medium, to which 10%, 20%, 30%, 40%, 50%, 60%, and 70% (w/v) glucose was added following the standard procedure [34–36]. Actively growing yeast colonies were inoculated into test tubes with various glucose concentrations and incubated at 30 °C for 48 h. Then, samples from 2-day-old fermentation broth (serial dilutions made following the methods mentioned in Sect. 2.3) were plated onto PDA plates using the spread plate technique and incubated again at 30 °C for 48 h. The number of viable cells on the respective sugar concentration was determined by counting colonies grown on PDA (HIMEDIA, India) containing g/L (Potatoes, infusion from, 200; Dextrose, 20; Agar 15).

2.5.4 Growth of yeasts at different salt concentrations

To examine the potential of yeast isolates to grow at different NaCl (Nice Chemicals, India), YEPD broth (HIMEDIA, India) containing g/L (yeast extract, 5; peptone, 5; D-glucose, 10) with a pH value of 5 was used. Then, 0.5% (w/v), 1% (w/v), 1.5% (w/v), and 2% (w/v) NaCl, was added to the broth separately. Selected yeast isolates containing an equal number of actively growing cells (approximately 3.6×10^6 CFU/mL) were inoculated into 50 mL flasks containing broth and incubated at 30 °C. After 24 h, the optical density at 550 nm was measured using a spectrophotometer (6405 UV/Vis, JENWAY, UK) [33] to determine cell growth.

2.5.5 Determination of agitation effect on yeast growth

YEPD broth (HIMEDIA, India) with g/L (yeast extract, 5; peptone, 5; D-glucose, 10) was used to examine the agitation effect on the growth of selected yeast isolates. Accordingly, approximately 3.6×10^6 cells mL^{-1} yeast cells were inoculated into 50 mL flasks containing YEPD broth and incubated at 30 °C in a shaking incubator (THZ-300C, China) at 140 rpm and non-shaking incubator (Model IN 150 C, China) simultaneously. After 4 days of incubation, the yeast cell density was measured at 550 nm using a spectrophotometer (6405 UV/Vis, JENWAY, United Kingdom) to compare the growth difference of the yeast cells incubated in the two incubators.

2.6 Starter culture formulation and determination of the leavening activity of selected yeast isolates

The potential leavening effect of selected yeast isolates was tested using wheat flour. Accordingly, a sample of wheat grain was washed with sterile distilled water and then sun-dried for a day. In a mill (Model BT-450, India), the dried grain was ground into a fine powder and sieved. After sterilizing the flour in an autoclave (Model AM B240, United Kingdom)

for one minute at 121 °C, the bread baking dough was prepared by combining 10 g of wheat flour, 0.09 g of table salt (Shewit salt, Ethiopia), and 0.45 g of table sugar (Nice Chemicals, India) with 20 mL sterile distilled water. After that, a loop-full of fresh colonies from each yeast isolate was inoculated into 10 mL of Sabouraud dextrose broth (HIMEDIA, India) containing g/L (peptone special, 10; dextrose, 20). The broth was incubated at 30 °C for 48 h and then centrifuged in a rotatory centrifuge (Model C3100, USA). Then, yeast isolates (about 9×10^6 CFU/mL determined through serial dilution), were suspended in 6 mL of distilled waterand were inoculated into samples of the dough mixture. The dough in each container was incubated at 25 °C (room temperature) and 37 °C for 0 h, 2 h, 4 h, 6 h, and 8 h. Dough not inoculated with yeast served as a negative control, while dough inoculated with commercial yeast served as a positive control. The dough volume was measured using a graduated cylinder (Model CYLINDER-11, China) before fermentation and every 2 h after fermentation [37]. The initial dough volume was 0.6 mL before incubation, similar to the negative control. Examining the effect of yeast on the raising of wheat dough was replicated three times by making the bread baking dough composition constant aimed to reduce experimental error. Finally, the volume of dough raised by each yeast isolate was recorded, and an average was taken for further computations.

2.7 Data analysis

The data were analyzed using the Minitab® 21 software program. The leavening activity of wheat dough by the selected yeast isolates was analyzed using ANOVA. The effects of temperature, pH, and salt concentration on yeast growth were examined using repeated measures analysis of variance. The graphs show the results of the pH tolerance test and the effect of agitation on yeast growth. Similarly, tables and bar charts were used to compare yeasts' capacity to exert a leavening effect in wheat dough incubated at 37 °C and room temperature.

3 Results

A total of 237 yeasts were isolated from *Teff*, maize, wheat, sorghum, finger millet, maize, barley, and bean sourdough. Among these isolates, 108 were identified as filamentous using cultural and microscopic techniques (Table S20). Out of all the yeast isolates tested for fermentation against eight sugar types, all filamentous yeasts (126) and 95 oval/circular yeasts were unable to ferment the majority of sugars except for glucose and fructose. Therefore, based on their morphological characteristics and sugar fermentation profiles, 16 yeast isolates that could ferment more than four sugar types were selected for further analysis. The results of these analyses are presented in the following sections.

3.1 Cultural characteristics of isolated yeasts

Sixteen yeasts were isolated from *Teff*, maize, wheat, sorghum, finger millet, maize and barley, sourdoughs. The color (varying from creamy to white), shape (oval, circular, or irregular), elevation (spread to elevated or convex), and texture (rough or smooth) varied among the yeast isolates' colonies. These characteristics are summarized in Table 1.

Table 1 Cultural characteristics of yeast isolates from fermented sourdough

Samples	Code and No_ of isolates per sample	Microscopic and colony Characteristics			
		Form/Shape	Color	Elevation	Margin
Sorghum	3 (Sor4, Sor8 and Sor9)	Oval/circular/	Creamy	Spread	Rough/smooth
Finger millet	1 (Fm23)	Circular	White	Raised	Rough
Barley	4 (Ba7, Ba9, Ba11 and Ba12	Circular	Creamy	Spread	Rough/smooth
Teff	2 (Te3 and Te19	Oval	White	Convex	Rough/smooth
Maize	2 (Maz1 and Maz2)	Oval	Creamy	Convex	Rough/smooth
Wheat	4 (Wh17, Wh5, Wh8 and Wh13)	Oval	White	Raised	Rough/smooth
Commercial yeast	1 (Cy)	Circular	White	Spread	Smooth

Cy commercial yeast (positive control), *Sor* Sorghum, *Fm* Finger millet, *Ba* Barley, Te *Teff*, *Maz* Maize, *Wh* Wheat

3.2 Carbohydrate fermentation test

All selected yeast isolates were fermented glucose, fructose, and sucrose. Like the commercial yeast (Cy), the Ba12, Wh17, Wh13, and Maz1 isolates also fermented xylose, while the remaining yeasts partially fermented this sugar. Unlike Cy, all the current yeast isolates partially fermented sorbitol. However, neither Cy nor the present isolates fermented lactose. In the test, a positive fermentation result was indicated by a yellow color, while a negative result was represented by a blue-black tint (the color of the base medium) (Fig s1). Based on the sugar fermentation test, the 16 best yeast isolates that fermented more than four types of sugar are presented in Table 2.

3.3 The effect of different temperatures on yeast growth

The ANOVA of the temperature tolerance test showed significant variation in the contribution of the temperature and incubation period at ($p < 0.05$) (Table S3).

The combined effect of temperature tolerance tests at temperatures of 27 °C, 32 °C, 37 °C, and 42 °C, incubated for 24 h, 48 h, and 72 h, confirmed which incubation periods were suitable for optimal growth of the yeast at the stated temperatures. Additionally, yeast isolates and of incubation period interaction effect indicated the influence of the incubation period on yeast growth. Therefore, Fm23, Maz1, Sor9, Wh13, and Wh15 isolates performed the best at 24 h and were statistically comparable to the Cy produced at 48 h. As a result, the commercial yeast performed better after 48 h, which represented a negligible change compared to the current best-performing isolates at 24 h. Wh15 and Maz2 isolates demonstrated comparable potential to Cy after 48 h of incubation. On the other hand, the least yeast growth was observed at 72 h, although substantial yeast growth occurred at 48 h (Table S4). Overall, 24 h of incubation produced the highest cell density, followed by 48 h of moderate yeast growth. The incubation period of 72 h was the least favorable to yeast proliferation (Table S5).

Results for the effects of temperature on yeast growth showed that 27 °C was ideal for yeast growth. Moderate yeast growth was observed even at temperatures of 32 °C and 37 °C, with no significant differences between the two temperatures. However, the lowest cell density was observed at 42 °C (Table S6). Most yeast isolates had temperature tolerance capabilities comparable to commercially available yeasts (Table S7).

Table 2 Sugar fermentation test of selected yeast isolates

Yeast isolates	Types of sugar							
	Glucose	Fructose	Sucrose	Maltose	Galactose	Xylose	Sorbitol	Lactose
Cy	+	+	+	+	+	+	+	−
Sor4	+	+	+	−	+	±	±	−
Sor8	+	+	+	+	+	±	±	−
Sor9	+	+	+	−	+	±	±	−
Fm23	+	+	+	+	+	±	±	−
Ba7	+	+	+	+	−	±	±	−
Ba9	+	+	+	+	+	±	±	−
Ba11	+	+	+	−	+	±	±	−
Ba12	+	+	+	+	+	+	±	−
Wh17	+	+	+	+	+	+	±	−
Wh5	+	+	+	+	−	±	±	−
Wh8	+	+	+	−	−	±	±	−
Wh13	+	+	+	−	+	+	±	−
Maz1	+	+	+	+	−	+	±	−
Maz2	+	+	+	+	+	±	±	−
Te3	+	+	+	+	+	±	±	−
Te19	+	+	+	−	+	±	±	−

+ indicates positive fermentation, − indicates no fermentation, ± indicates partial fermentation, *Cy* commercial yeast (positive control), *Sor* Sorghum, *Fm* Finger millet, *Ba* Barley, Te *Teff*, *Maz* Maize, *Wh* Wheat

The interaction effect of the incubation period (IP) and temperature showed that incubation at 27 °C, 32 °C, and 37 °C for one day and at 27 °C for two days was the most favorable conditions for yeast growth, with mean scores of 0.85 to 0.96. Sufficient yeast growth was also detected at 32 °C after 48 h of incubation, with a mean score of 0.69. Statistically similar yeast growth was observed when yeast was incubated at 37 °C for 48 h and at 27 °C, 32 °C, and 37 °C for 72 h, with mean scores ranging from 0.55 to 0.63. In contrast, yeast isolates incubated at 42 °C for 24 h and 48 h showed inhibited growth, with mean scores of 0.28 and 0.25, respectively. Incubation at 42 °C for 48 h and 72 h was an unfavorable condition characterized by limited and inhibited yeast growth, which was confirmed by the lowest OD record. Moreover, no yeast growth was recorded when incubated at 42 °C for three days, as confirmed by a mean OD score of less than 0.2 (Table S8).

3.4 Growth of yeasts at different pH values

The pH values had the highest influence on the overall variability of the pH tolerance test. Most yeast isolates showed the highest optical density (OD) at pH 4, the ideal pH for yeast growth. Unlike the other isolates, Sor8 grew fastest at pH 6. As the pH of the medium in which the yeast isolates grew raised to 5 and 6, the OD decreased. In other words, as the pH increased, the OD of most yeast isolates decreased, showing that pH and OD are inversely related (Fig. 1). The optimal pH for yeast growth was pH 4, followed by pH 5 and 6. The overall pH tolerance assessment revealed that Wh17 was one of the yeast isolates with the highest pH tolerance.

3.5 Glucose tolerance and carbon dioxide production test of yeasts

Table 3 shows the tolerance potential of the selected yeast isolates at various glucose concentrations and CO_2 production conditions. All yeast isolates grew effectively in the presence of 30% glucose. However, the yeast isolate Fm23 failed to grow and was resistant to more than 40% glucose, as evidenced by the formation of fewer than 30 colonies on the SDA medium. Except for Sor8, Te3, and Te19, the remaining yeast isolates were 50% glucose tolerant. Ba7, Ba9, Ba11, Ba12, Wh17, Wh5, Wh8, Wh13, Maz1, and Maz2 were the most resistant isolates to osmotic stress with 60% glucose, similar to commercial yeasts. However, no yeast isolates grew on 70% glucose, as evidenced by the almost complete absence of colonies on the agar plate. In general, the growth of 30–300 colonies on SDA confirmed positive growth at a given glucose concentration, while fewer than 30 colonies confirmed negative growth.

All yeast isolates except Fm23 produced CO_2 gas after 24 h of incubation, which was confirmed by the formation of bubbles in YEPD broth (HIMEDIA, India) containing g/L (yeast extract, 5; peptone, 5; D-glucose, 10). The results of temperature and CO_2 production test results are presented in Table 3.

3.6 Growth of yeasts at different salt concentrations

Selected yeast isolates were tested for their salt tolerance at different NaCl concentrations (% w/v). The results showed that yeast growth was maximal at 0.5% NaCl and decreased as the NaCl concentration increased up to 2%.

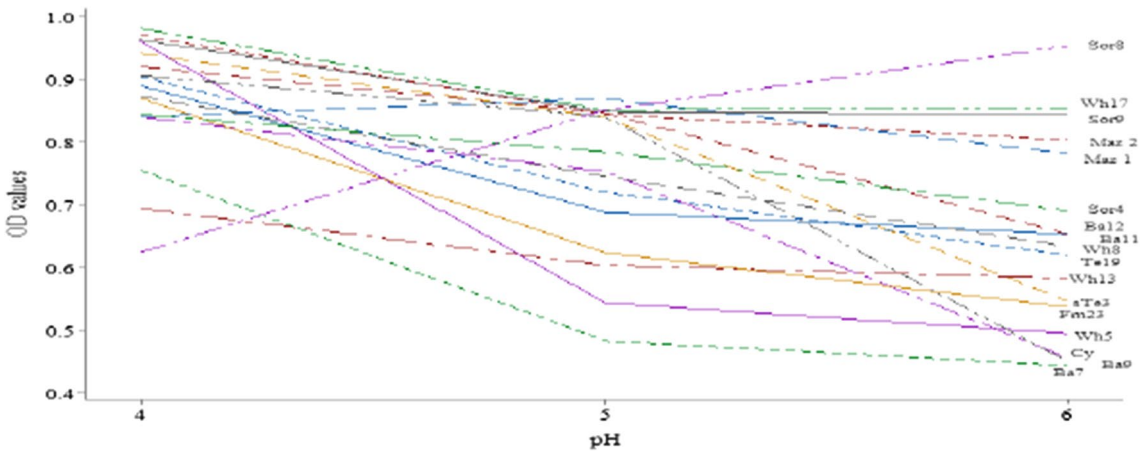

Fig. 1 Growth of yeast isolates at different pH values. *Cy* commercial yeast (positive control), *Sor* Sorghum, *Fm* Finger millet, *Ba* Barley, Te *Teff*, *Maz* Maize, *Wh* Wheat

Table 3 Glucose tolerance and carbon dioxide production status for selected yeast isolates

Yeast isolates	Glucose concentration							Co_2 production
	10	20	30	40	50	60	70	+
Cy	+	+	+	+	+	+	–	+
Sor4	+	+	+	+	+	–	–	+
Sor8	+	+	+	+	-	–	–	+
Sor9	+	+	+	+	+	–	–	+
Fm23	+	+	+	–	–	–	–	–
Ba7	+	+	+	+	+	+	–	+
Ba9	+	+	+	+	+	+	–	+
Ba11	+	+	+	+	+	+	–	+
Ba12	+	+	+	+	+	+	–	+
Wh17	+	+	+	+	+	+	–	+
Wh5	+	+	+	+	+	+	–	+
Wh8	+	+	+	+	+	+	–	+
Wh13	+	+	+	+	+	+	–	+
Maz 1	+	+	+	+	+	+	–	+
Maz 2	+	+	+	+	+	+	–	+
Te3	+	+	+	+	–	–	–	+
Te19	+	+	+	+	–	–	–	+

+ well growth in the specified sugar concentration (30–300 colonies), – No growth in the specified sugar concentration (< 30 colonies); *Cy* commercial yeast (positive control), *Sor* Sorghum, *Fm* Finger millet, *Ba* Barley, Te *Teff*, *Maz* Maize, *Wh* Wheat

Although the total OD recording was maximal at 0.5% NaCl, the yeast cell concentration grown at 1% and 1.5% was not negligible. However, as the salinity increased, the yeast growth rate correspondingly decreased, indicating an inverse relationship between salt concentration and yeast growth (Fig. 2). The salt tolerance experiment showed that Wh13, Ba7, Cy, Sor4, Ba11, Ba9, Wh17, Wh5, Maz 2, Ba12, Te3, Maz 1, Fm23, Wh8, and Sor8 had the highest salt tolerance capacity. These isolates had no significantly different salt tolerance capacities, and most of the yeasts in this study had statistically similar salt tolerance capacities to commercial yeasts (Table S14).

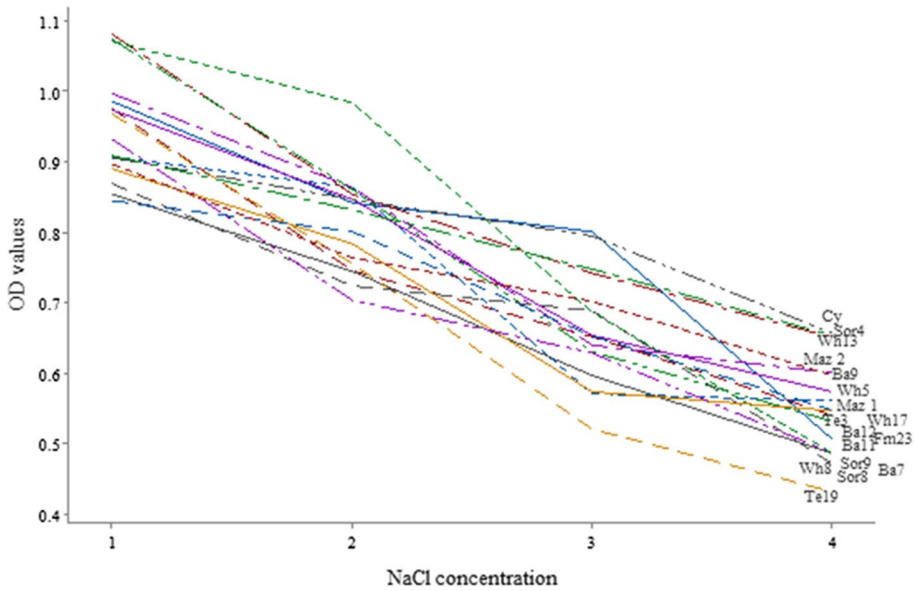

Fig. 2 NaCl tolerance level of yeast isolates. *Cy* commercial yeast (positive control), *Sor* Sorghum, *Fm* Finger millet, *Ba* Barley, Te *Teff*, *Maz* Maize, *Wh* Wheat

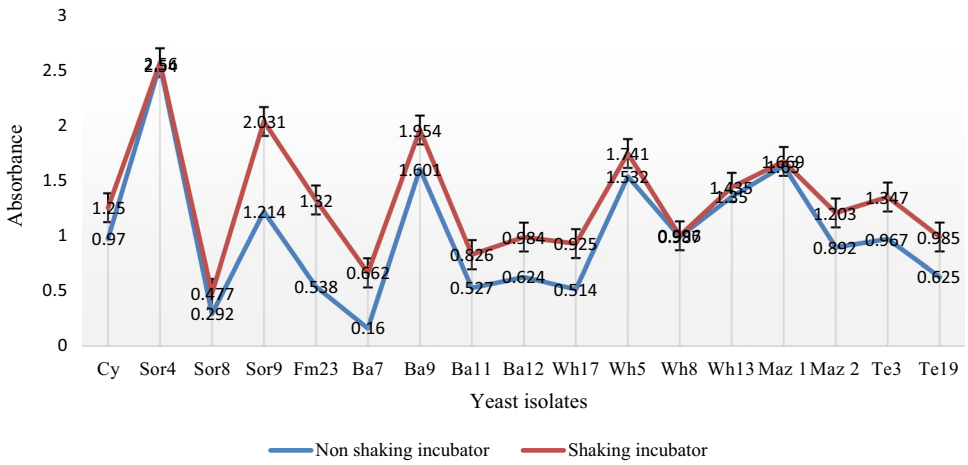

Fig. 3 Effect of agitation on the growth of yeast isolates. *Cy* commercial yeast (positive control), *Sor* Sorghum, *Fm* Finger millet, *Ba* Barley, Te *Teff*, *Maz* Maize, *Wh* Wheat

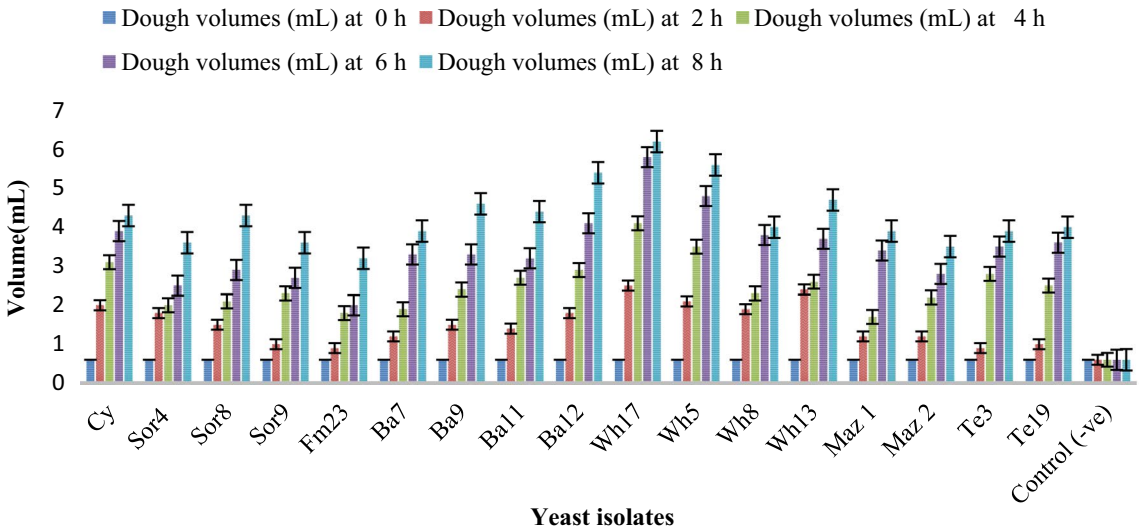

Fig. 4 Mean effect of different yeast isolates in the leavening activity of wheat flour dough incubated at 37 °C. *Cy* commercial yeast (positive control), *Sor* Sorghum, *Fm* Finger millet, *Ba* Barley, Te *Teff*, *Maz* Maize, *Wh* Wheat, *VC* –ve negative control

3.7 Determination of agitation effect on yeast growth

The yeast isolates were compared for their growth potential in incubators with and without shaking. Higher absorbance was recorded in the shaking incubator (Model THZ-300C, China) compared to the non-shaking incubator (Model IN 150C, China), indicating higher yeast density. Furthermore, yeast cell density decreased from commercial yeast to *Teff*-derived yeast (Te19) in both incubators with and without shaking, although the highest record was achieved by Sor4, among others (Fig. 3).

3.8 Starter culture formulation and determination of the leavening activity of selected yeast isolates

The leavening activity of selected potent yeast isolates in wheat dough was evaluated and compared at 37 °C and room temperature based on the mean dough volume. In both experiments, the leavening activity increased with incubation time (Figs. 4 and 5). The highest mean dough volume was recorded at the 8th hour for both obtained from wheat dough-derived yeast isolates (Wh5 and Wh17). The lowest dough volume was recorded at the 8th hour for the isolate Fm23 at both temperatures. All samples, except the negative control, showed an increase in dough volume.

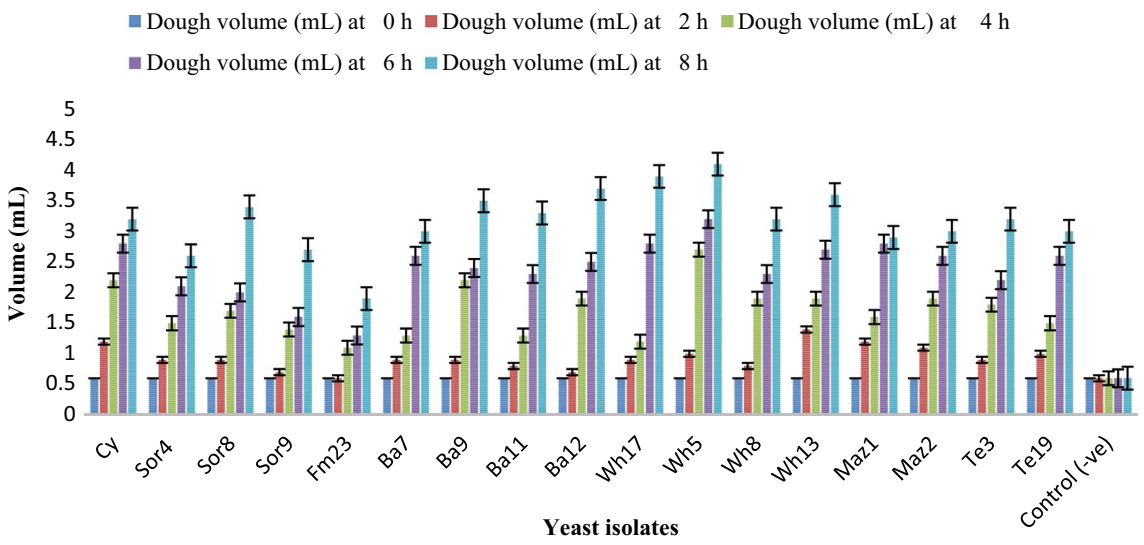

Fig. 5 Mean effect of different yeast isolates in the leavening activity of wheat flour dough incubated at room temperature. *Cy* commercial yeast (positive control), *Sor* Sorghum, *Fm* Finger millet, *Ba* Barley, Te *Teff*, *Maz* Maize, *Wh* Wheat, *VC* –ve negative control

Low leavening activity was observed in the negative control (0.6 mL), which remained unchanged throughout the incubation period.

The highest dough volume (6.2 mL) was recorded when the dough was inoculated with yeast isolate Wh17 and incubated at 37 °C, which was the highest volume compared to all other samples, including the dough inoculated with the commercial yeast. Conversely, the lowest dough volume (3.2 mL) at the 8th hour was recorded for the isolate Fm23 (Fig. 4). At room temperature, the highest dough volume (4.1 mL) was recorded when Wh5 was inoculated into the dough. Contrariwise, the lowest dough volume (1.9 mL) was recorded for the dough inoculated with the yeast isolate Fm23 (Fig. 5).

Analysis of variance (ANOVA) of the leavening activity when incubated at 37 °C and room temperature revealed a highly significant variation ($p < 0.001$) among yeast, time, temperature, and their respective interactions, indicating the main effect of these factors on the experiment, as confirmed based on their statistical significance with a confidence level of 99.95% (Table S9).

The differences between or among the means of the leavening activity of wheat dough by selected yeast isolates were evaluated. As a result, Wh17, Wh5, and Ba12 were the top-ranked potent yeast isolates that significantly increased the volume of wheat dough compared to the control strain (Cy). In contrast, the potential of isolates Ba12, Wh13, and Ba9 was statistically non-significant compared to the strain Cy, while isolates Ba7, Sor4, Sor9, and Fm23 had the least potential in the leavening activity of the dough (Table 4) and (TableS11). The leavening activity or volume of the dough increased over time from 0 to 8 h, both at 37 °C and at room temperature. For example, the highest dough volume was recorded by Wh17 at 37 °C and 8 h of incubation. In general, the leavening activity was increased as the incubation hours increased (8 h > 6 h > 4 h > 2 h > 0 h). At the significance level ($p < 0.05$), the t-test revealed a significant difference between the dough volume incubated at 37 °C and room temperature, indicating that the leavening potential was significantly higher at 37 oC than at room temperature (Table S18).

3.9 Optimum growth and fermentation conditions for the selected yeast isolates

The different growth and fermentation parameters under which yeasts respond positively or perform better, including the types of sugar they ferment, their osmotic tolerance, optimum temperature, pH, IP, and NaCl concentration, are presented. Additionally, optimized conditions where the wild yeast isolated from Tigray, Ethiopia sourdough produce higher dough volume are presented in Table 5.

Table 4 Interaction effect of yeast isolates, IP, and temperature in the leavening activity of wheat dough

Yeast isolates	IP (h)	Mean dough volume (mL)	
		37 °C	Room temp
Ba11	0	0.6 ± 0	0.6 ± 0
	2	1.4 ± 0.53	0.8 ± 0.2
	4	2.7 ± 0.26	1.3 ± 0.1
	6	3.2 ± 0.26	2.3 ± 0.26
	8	4.4 ± 0.53	3.3 ± 0.26
Ba12	0	0.6 ± 0	0.6 ± 0
	2	1.8 ± 0.26	0.7 ± 0.1
	4	2.9 ± 0.26	1.9 ± 0.2
	6	4.1 ± 0.2	2.5 ± 0.2
	8	5.4 ± 0.2	3.7 ± 0.2
Ba7	0	0.6 ± 0	0.6 ± 0
	2	1.2 ± 0.1	0.9 ± 0.1
	4	1.9 ± 0.2	1.3 ± 0.17
	6	3.3 ± 0.2	2.6 ± 0.1
	8	3.9 ± 0.2	3 ± 0.26
Ba9	0	0.6 ± 0	0.6 ± 0
	2	1.5 ± 0.26	0.9 ± 0.3
	4	2.4 ± 0.17	2.2 ± 0.1
	6	3.3 ± 0.26	2.4 ± 0.3
	8	4.6 ± 0.2	3.5 ± 0.1
Cy	0	0.6 ± 0	0.6 ± 0
	2	2 + 0.87	1.2 ± 0.26
	4	3.1 ± 0.26	2.2 ± 0.1
	6	3.9 ± 0.17	2.8 ± 0.1
	8	4.3 ± 0.36	3.2 ± 0.26
Fm23	0	0.6 ± 0	0.6 ± 0
	2	0.9 ± 0.26	0.6 ± 0
	4	1.8 ± 0.1	1.1 ± 0.1
	6	2 ± 0.26	1.3 ± 0.1
	8	3.2 ± 0.26	1.9 ± 0.2
Maz 1	0	0.6 ± 0	0.6 ± 0
	2	1.2 ± 0.1	1.2 ± 0.1
	4	1.7 ± 0.46	1.6 ± 0.1
	6	3.4 ± 0.43	2.8 ± 0.1
	8	3.9 ± 0.62	2.9 ± 0.17
Maz 2	0	0.6 ± 0	0.6 ± 0
	2	1.2 ± 0.2	1.1 ± 0.17
	4	2.2 ± 0.26	1.9 ± 0.2
	6	2.8 ± 0.36	2.6 ± 0.26
	8	3.5 ± 0.2	3 ± 0.1
Sor4		0.6 ± 0	0.6 ± 0
	2	1.8 ± 0.2	0.9 ± 0.26
	4	2 ± 0.1	1.5 ± ,1
	6	2.5 ± 0.61	2.1 ± 0.36
	8	3.6 ± 0.26	2.6 ± 0.17
Sor8	0	0.6 ± 0	0.6 ± 0
	2	1.5 ± 0.17	0.9 ± 0.2
	4	2.1 ± 0.1	1.7 ± 0.43
	6	2.9 ± 0.61	2 ± 0.1

Table 4 (continued)

Yeast isolates	IP (h)	Mean dough volume (mL)	
		37 °C	Room temp
	8	4.3 ± 0.2	3.4 ± 0.1
Sor9	0	0.6 ± 0	0.6 ± 0
	2	1 ± 0.1	0.7 ± 0.1
	4	2.3 ± 0.1	1.4 ± 0.17
	6	2.7 ± 0.26	1.6 ± 0.26
	8	3.6 ± 0.3	2.7 ± 0.2
Te19	0	0.6 ± 0	0.6 ± 0
	2	1 ± 0.1	1 ± 0.1
	4	2.5 ± 0.4	1.5 ± 0.1
	6	3.6 ± 0.26	2.6 ± 0.17
	8	4 ± 0.26	3 ± 0.3
Te3	0	0.6 ± 0	0.6 ± 0
	2	0.9 ± 0.1	0.9 ± 0.2
	4	2.8 ± 0.36	1.8 ± 0.2
	6	3.5 ± 0.4	2.2 ± 0.17
	8	3.9 ± 0.46	3.2 ± 0.4
VC	0	0.6 ± 0	0.6 ± 0
	2	0.6 ± 0	0.6 ± 0
	4	0.6 ± 0	0.6 ± 0
	6	0.6 ± 0	0.6 ± 0
	8	0.6 ± 0	0.6 ± 0
Wh13	0	0.6 ± 0	0.6 ± 0
	2	2.4 ± 0.1	1.4 ± 0.17
	4	2.6 ± 0.53	1.9 ± 0.4
	6	3.7 ± 0.4	2.7 ± 0.2
	8	4.7 ± 0.56	3.6 ± 0.2
Wh17	0	0.6 ± 0	0.6 ± 0
	2	2.5 ± 0.3	0.9 ± 0.1
	4	4.1 ± 0.2	1.2 ± 0.1
	6	5.8 ± 0.3	2.8 ± 0.36
	8	6.2 ± 1.04	3.9 ± 0.3
Wh5	0	0.6 ± 0	0.6 ± 0
	2	2.1 ± 0.1	0.97 ± 0.15
	4	3.5 ± 0.44	2.7 ± 0.17
	6	4.8 ± 0.1	3.2 ± 0.1
	8	5.6 ± 0.26	4.1 ± 0.3
Wh8	0	0.6 ± 0	0.6 ± 0
	2	1.9 ± 0.4	0.8 ± 0.1
	4	2.3 ± 0.1	1.9 ± 0.36
	6	3.8 ± 0.4	2.3 ± 0.26
	8	4 ± 0.35	3.2 ± 0.1

Cy commercial yeast (positive control), *Sor* Sorghum, *Fm* Finger millet, *Ba* Barley, Te *Teff*, *Maz* Maize, *Wh* Wheat, *VC* –ve negative control

4 Discussion

Based on the morphological and microscopic characterization (Table S20), the yeasts involved in the current study can be assigned to the genus *Saccharomyces* and the *Candida humilis*. A previous study by Tadesse et al. [38] reported

Table 5 The optimum growth and fermentation conditions for the selected yeast isolates

Yeast isolates	Types of carbohydrate and positively fermented for	Biochemical tests					Favorable leavening activity at 37 °C	
		Glucose tolerance (%)	Temp (°C)	IP	pH	NaCl (%)	IP (h)	Dough volume (mL)
Cy	Glu, Fru, Suc, Mal, Gal, Xyl, Sor	10–60	37	48	4	0.5	8	4.3
Sor4	Glu, Fru, Suc, Gal	10–50	32	48	4	0.5	8	3.6
Sor8	Glu, Fru, Suc, Mal, Gal	10–40	27, 37	24	6	0.5	8	4.3
Sor9	Glu, Fru, Suc, Gal	10–50	27, 37	24	4	0.5	8	3.6
Fm23	Glu, Fru, Suc, Mal, Gal	10–30	27, 37	24	4	0.5	8	3.2
Ba7	Glu, Fru, Suc, Mal	10–60	27	48	4	0.5	8	3.9
Ba9	Glu, Fru, Suc, Mal, Gal	10–60	27	48	4	0.5	8	4.6
Ba11	Glu, Fru, Suc, Gal	10–60	27	48	4	0.5	8	4.4
Ba12	Glu, Fru, Suc, Mal, Gal, Xyl	10–60	27	48	4	0.5	6	5.4
Wh17	Glu, Fru, Suc, Mal, Gal, Xyl	10–60	27, 32	24	4	0.5	8, 6	6.2
Wh5	Glu, Fru, Suc, Mal	10–60	37	24	4	0.5	8	5.6
Wh8	Glu, Fru, Suc	10–60	27	48	4	0.5	8	4.0
Wh13	Glu, Fru, Suc, Gal, Xyl	10–60	27, 32	24	4	0.5	8	4.7
Maz1	Glu, Fru, Suc, Mal, Xyl	10–60	27, 32	24	5	0.5	8	3.9
Maz2	Glu, Fru, Suc, Mal, Gal	10–60	27, 32	24	4	0.5	8	3.5
Te3	Glu, Fru, Suc, Mal, Gal	10–40	27	48	4	0.5	8	3.9
Te19	Glu, Fru, Suc, Gal	10–40	27	48	4	0.5	8	4.0

Glu Glucose, *Fru* Fructose, *Suc* Sucrose, *Mal* Maltose, *Gal* Galactose, *Xyl* Xylose, *Sor* Sorbitol, *Lac* Lactose, *Cy* commercial yeast (positive control), *Sor* Sorghum, *Fm* Finger millet, *Ba* Barley, Te *Teff*, *Maz* Maize, *Wh* Wheat

that *Candida humilis was* white and circular with a flat elevation that was almost similar to current yeast isolates. Hence, these yeasts could be the dominant yeasts and play a vital role in the fermentation of sourdough from the traditional grain dough found in Tigray, Ethiopia.

The biochemical characteristics of the current yeast isolates resemble *C. humilis*. Muche et al. [39] reported that *S. cerevisiae* and *C.humilis* were found in Ethiopian sourdough Injera. They found that two *C.humilis* (G3N1 & B6N3) where G3N1 had creamy colony color; both G3N1 and B6N3 had better growth at 37 °C and pH 4.2, resisted osmotic stress, and grew well at different glucose concentrations. Moreover, both strains fermented glucose, sucrose, galactose, and maltose as a carbon source except lactose. Furthermore, *C. humilis* is a good carbon oxide producer and is one of the dominant yeast in Ethiopian fermented dough [38]. *C. humilis* is one of the indigenous wild yeasts studied in sour dough, and potent strains showed increased biomass yield and impressive leavening power [40]. The biochemical characteristics of the present yeast isolates discussed in the following paragraphs are almost similar to *C. humilis* and *S. cerevisiae*.

In the present study, all sourdough yeast isolates were excellent in fermenting various sugars. These could be due to the isolation of these yeasts from the sugar-rich dough of these grains. These yeasts were then grown by consuming and adapting the sugars present in the sourdough. This consequently causes the yeasts to break down the sugars contained in the dough, such as glucose, fructose, sucrose, maltose, galactose, and xylose. The presence of these yeast isolates in the sugar changes the color of the basal medium from blue-black to yellow. A previous study by James and Barnett [41] reported that the color change of the medium to yellow during sugar fermentation was due to acid production, confirming positive fermentation. In this study, the isolates are categorized into the genus *Saccharomyces*. The previous findings of Nasir et al. [42] reported that a yeast strain that fails to ferment lactose but ferments glucose, sucrose, maltose, fructose, galactose, and xylose is considered a strain of *S. cerevisiae*. However, none of the yeast isolates were able to ferment the sugar lactose. Olowonibi [43] stated that yeasts' failure to ferment lactose was due to a deficiency of the enzyme lactase. The ability of yeast to ferment many types of sugars indicates that the isolates contain enzymes that hydrolyze the starch formed as it leaves the dough. Most yeast isolates were good producers of carbon dioxide, which resulted from the fermentation of the starch contained in the flour. It is also helpful to increase the dough volume during fermentation. The carbon dioxide produced during fermentation is used to soften the bread and helps the dough to rise before baking.

The salt tolerance test in the current study revealed that the yeast cell viability decreased with increasing salt concentration, which is consistent with the reports by Kechkar et al. [44] and Goston et al. [45], who found that higher salinity damaged yeast cells and resulted in impaired cellular functions. Navarrete et al. [46] also reported that high concentrations of NaCl were toxic to yeast cells and led to a slowdown in their growth. Yeast cells release a portion of their water to the salt via osmosis when exposed to high salt concentrations. This consequently results in a delay in the reproduction or duplication of the yeasts and results in reduced fermentation. Furthermore, Luchian and Canja [47] stated that excess salt in bread retarded the yeasts to the point where their volume decreased, leading to a corresponding reduction in fermentation. Thus, the current yeast isolates can exhibit excellent fermentation/leavening activity at standard salt concentrations.

In the present study, the temperatures favorable for yeast growth were between 27 and 37 °C, and these ranges may be suitable for the effective action of these yeast enzymes. It is believed that strong yeast can grow at temperatures of 37 °C. Li et al. [48] described that yeasts with an optimal growth temperature below 38 °C provide optimal conditions for increased enzymatic activity and are useful in the hydrolysis of sugars. However, in the current study, complete inhibition of yeast cells was observed at 42 °C. This may be due to the inability of yeast cells to withstand this temperature and can denature in this range. The new findings were superior to those of the earlier study by Ali and Khan [49], who demonstrated that the growth of the yeast strain was completely inhibited in the temperature range of 37 to 45 °C. The researchers hypothesized that the inhibition was due to the denaturation of the tertiary structure of the yeast proteins and that the tertiary structure of the proteins in these isolates was denatured and no longer functional.

In the present study, a significantly higher yeast cell density was observed at pH 4, which was confirmed by most previous studies. According to Johnston et al. [50], the ideal pH for the growth of *S. cerevisiae* is between 4 and 6, and this pH range also aids in dealing with contaminants. For this reason, changing the pH of the medium to 4 is beneficial when kneading large amounts of dough, which is vital in the baking industry.

In the current finding, most yeast isolates cultured on conditions containing up to 60% sugar were naturally resistant to sugar stress. Hence, the present yeast isolates showed improved glucose tolerance capacity compared to the four yeast strains [CICY-RN-035 (*Metschnikowia koreensis*), CICY-RN-209 (*Sympodiomycopsis paphiopedili*), CICY-RN-364 (*Starmerella apicola*) and CICY-RN- 354 (*Starmerella apicola*)], which showed a significant decrease in cell density at a sugar concentration of 60% [51]. Yeast strains capable of thriving at relatively high sugar concentrations have proved their ability to endure osmotic stress by activating the high-osmolar glycerol pathway and many transcription factors responsible for the response to high sugar concentrations [52]. The difficulties in growing yeasts in the presence of 70% sugar may be related to their inability to overcome the osmotic stress caused by glucose. Varelas et al. [53] previously reported that high sugar concentrations expose yeast cells to osmotic pressure, leading to a reduction in glucan synthesis during fermentation. This reduced the sugar consumption of the yeasts and their ability to ferment substrates [54]. According to Marques et al. [55], *S. cerevisiae,* produced from sugar-rich substrates, can efficiently absorb sugars such as sucrose, making it one of the most essential cells for stress tolerance. Most of the yeast isolates considered in this study, particularly those with glucose tolerance up to 60%, are appropriate candidates for fermentation of wheat flour, potentially offering good qualities for use in the baking industry. As a result, they could be suitable strains for other stress-tolerant yeasts needed in the food industry to produce nutritious foods from sugar/starch-rich substrates. Since the yeasts examined in this study thrived effectively in a shaking incubator, bioreactors with rotatory objects may need to be designed or locally modified to distribute nutrients among yeast cells during fermentation. It can be beneficial for the yeast to spread throughout the substrate in which it is fermenting for maximum efficiency.

In the present study, the poor leavening activity of the negative control, which was yeast-free dough, corroborated the influence of yeast on the fermented dough. The yeast isolates used in this study were well fermented for typical wheat flour. As a result of the leavening activity, the experiment demonstrated the ability of the yeasts to use sugars and other nutrients in wheat flour to generate gas and raise the dough. This means that the carbon dioxide released during fermentation can cause the dough to increase in volume. Yeast converts the fermentable carbohydrates in the dough into carbon dioxide, allowing the dough to expand in volume as the CO_2 bubbles during fermentation [54]. The specific sucrase enzyme produced by prospective yeasts plays a vital role in dough fermentation [56]. *S. cerevisiae* and *Candida humilis* are among the most common yeasts in Ethiopian fermented foods, demonstrating that these yeasts represent the primary yeast microbiota in sourdough [57]. When yeasts are added to the dough, they begin fermentation, absorbing sugars from the dough and releasing CO_2 and other compounds that give the baked product its flavor and scent. Therefore, *Saccharomyces* and the *Candida humilis* can impart taste to the locally made *Injera* and specific bread made from the specified grains. The current discovery could help produce standard *Injera*, Ethiopia's most fermented flour.

According to Qvirist et al. [32], an optical density (OD) below 0.2 indicates no yeast growth; an OD between 0.2 and 0.4 indicates positive but inhibited growth and an OD greater than 0.4 indicates positive growth. In the present study, potential isolates were grown at 37 °C and incubated for 24 h or 48 h. For example, the potent isolates Fm23, Maz1, Sor9, Wh15, and Wh13, which can complete their growth/reproduction within 24 h, were confirmed by their high OD values, indicating that high yeast growth is preferable. These yeasts can have a rapid reproduction or doubling rate, allowing them to achieve optimal growth within 24 h compared to other yeasts that require 48 h.

Compared to the commercial yeasts that require 48 h to reproduce at high levels, the current yeast isolates had a remarkable ability to reproduce at high levels within 24 h, outperforming four yeast strains (*Wickerhamomyces anomalus*, *Saccharomycopsis fibuligera*, *Papiliotrema flavescens*, and *Pichia kudriavzevii*), with the maximum cell density observed at 48 h of fermentation [58]. Therefore, the present yeast isolates can make dough rise or convert sugary raw materials into products within 24 h, reducing the time required for the fermentation process by a day. As a result, significant dough volume was recorded during the 8 h incubation with these yeast isolates.

Yeast isolates grown within 24 h at room temperature can solve two problems: they reduce the energy required to increase the temperature to ≥ 37 °C while enabling the conversion of substrates into products in less than 48 h. For example, the yeast isolate Wh17 showed the best leavening activity when incubated at 37 °C for less than 24 h. A previous study showed that the amount of CO_2 produced by baker's yeast at high temperatures was higher than at room temperature, but CO_2 production increased much faster in yeasts exposed to 29 °C compared to 35 °C [59]. Therefore, the current yeast isolates are the best candidates for performing various starch processing functions at 37 °C and at room temperature.

Studies showed that sourdough yeasts would be superior to commercial yeast when making bread since the bread would have a greater protein content, a longer shelf life, and the needed color, texture, taste, odor, and general acceptability [60]. Hence, sourdough-adapted yeast metabolism mostly contributes to the leavening and flavor of sourdough goods from a technological standpoint. Aside from ethanol and carbon dioxide, yeasts can create metabolites that directly alter flavor, such as organic acids, diacetyl, higher alcohols, and esters [61]. Additionally, yeasts isolated from sourdough are stress-tolerant and potentially a leavening agent in the leavening dough, exceeding the capacity of commercial baker's *S. cerevisiae* [62], characteristics of the current yeast isolates. Likewise, sourdough yeast strains have functional features that can potentially lead to nutritional and safety benefits. These qualities include vitamin synthesis, increased bioavailability of phenolic compounds, phytic acid dephosphorylation, the presence of probiotic potential, and suppression of fungus and mycotoxin development [61]. As confirmed in the present study, wild yeasts can be obtained from cheap resources that everyone can access. However, it is necessary to identify factors affecting the fabrication and effective method of dehydration of sourdough-originated yeast considering its fermentation complexity [63]. Henceforward, it is necessary to develop farmer-friendly production methodology and technology and obtain dehydrated sourdough starters for domestic use in local supermarkets. The current yeast isolates can be valuable in the bakery sector and for use at home in places like Ethiopia, where Injera is the main meal. That is important to optimize grain dough fermentation and generate consistent food products with excellent nutritional content. That facilitates the production of fermented products in a timely and cost-effective manner.

5 Conclusion

Overall, selected yeasts isolated from *Teff*, maize, wheat, sorghum, finger millet, and barley sourdough showed that they were capable of fermenting a variety of sugars including glucose, fructose, sucrose, maltose, and galactose, withstanding high glucose concentrations (up to 60%), and producing carbon dioxide are crucial candidates for leavening wheat flour. Additionally, pH 4, 0.5–1% of NaCl, and temperature of 27–37 °C incubated for 24–48 h were the most favorable conditions for the growth of most yeast isolates. In the leavening activity, Wh17, Wh5, and Ba12 were the top-ranked potent yeast isolates that significantly increased the volume of wheat dough at 37 °C than room temperature. Unlike commercial yeasts, this study discovered extraordinary yeasts that mature within 24 h. Therefore, the current yeast isolates can produce yeast dough or convert sugary raw materials into products within 24 h, reducing the time required for commercial yeasts by one day. The current yeast isolates can have a rapid doubling rate and be cost-effective because they can reduce substrate and energy consumption. Hence, yeast can be used to ferment various grain substrates, making them suitable for food and other industrial processes that require yeast. Additionally, the present study demonstrated the potential of fermented sourdough in grains as a potential source of yeasts for the baking industry. Furthermore, the yeasts are intended to be used as starter cultures in homemade bread and baked goods, particularly in rural villages where *Injera* and traditional bread are the staple meals. Sourdough-adapted yeast metabolism contributes to the leavening and flavor

of sourdough goods from technological a standpoint. However, it is necessary to identify factors affecting the fabrication and effective method of dehydration of sourdough-originated yeast considering its fermentation complexity. Further studies are recommended to investigate wild yeasts at the molecular level.

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Author contributions Sample collection, conceptualization, investigation, writing—original draft and visualization were performed by GTA, MMK, ADB, and ABK. YTR and MYW achieved validation and project administration. YTB and MMM performed data analysis and interpretation. TSG, LTW, HTM, ATY, EME, SZH, HMG, and BKM accomplished writing review and editing, and supervision. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable

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