



# Microbial dynamic and growth potential of selected pathogens in Ethiopian traditional fermented beverages

Reda Nemo<sup>1,2</sup> and Ketema Bacha<sup>1\*</sup>

## Abstract

**Purpose:** The patterns of microbial succession and the associated physicochemical changes in the course of beverage fermentation determine the safety status of the final product against foodborne pathogens. In this study, the microbial dynamics during fermentation of three Ethiopian traditional fermented beverages (namely, borde, tej, and grawa) and the growth potential of selected foodborne pathogens in ready-to-consume beverages were assessed.

**Methods:** The raw materials used for lab-scale fermentation of the beverages were bought from open markets of Jimma and Anfilo towns. During fermentation, samples were drawn every 6 h (borde fermentation) and 12 h (grawa and tej fermentation). The dominant microbes of the fermentation phases were determined following standard microbiological methods. The growth potential of *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Candida albicans* in the ready-to-consume beverages were assessed by microbial enumeration over defined storage period.

**Result:** Early fermentation period of all beverages was dominated by aerobic mesophilic bacteria, staphylococci, and Enterobacteriaceae with highest mean counts (Log CFU/ml) of  $6.42 \pm 0.10$ ,  $5.44 \pm 0.08$ , and  $5.40 \pm 0.11$ , respectively. At the end of fermentation, yeast counts (Log CFU/ml) dominated in tej ( $9.41 \pm 0.06$ ) and grawa ( $7.88 \pm 0.02$ ) samples, while lactic acid bacteria dominated in borde sample ( $7.33 \pm 0.07$ ). During fermentation, pH dropped for borde ( $4.58 \pm 0.03$  to  $4.22 \pm 0.01$ ), and grawa ( $4.18 \pm 0.10$  to  $3.62 \pm 0.02$ ), but increased for tej ( $5.26 \pm 0.01$  to  $5.50 \pm 0.03$ ) during the first 24 h, though it dropped later down to  $3.81 \pm 0.02$  at 144th h. All reference pathogens were unable to reach infective dose in grawa and tej samples. However, borde sample supported their growth to infective dose within 24 h. Thus, grawa and tej beverages had the capability of inhibiting growth of pathogens while borde needs basic safety control measures during preparation and storage.

**Conclusion:** With further safety evaluation of the products, the production processes of the three beverages could be scaled up for commercial purposes using defined starter cultures originated from the same beverages. However, the safety status of borde calls for further evaluation for alternative shelf-life extension mechanisms including the introduction of organic preservatives from local products such as medicinal plants.

**Keywords:** Beverages, Fermentation, Growth potential, Microbial dynamic, Pathogens

\* Correspondence: [ketemabacha2002@yahoo.com](mailto:ketemabacha2002@yahoo.com)

<sup>1</sup>Department of Biology, College of Natural Sciences, Jimma University, P.O.Box 378, Jimma, Ethiopia

Full list of author information is available at the end of the article



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## Highlights

- LAB and yeast dominated the fermentation of all local beverages.
- The counts of molds, coliform, and Enterobacteriaceae were below detectable level at the end of fermentation.
- *Tej* and *grawa* beverages did not support the growth of test pathogens but *borde*.
- *E. coli* and *S. typhimurium* showed higher survival ability than *S. aureus* and *L. monocytogenes* in *grawa* and *tej* samples.
- The beverage could be source of probiotic and starter culture LAB and yeasts.

## Introduction

Fermentation is a low-priced food biotechnological process being used worldwide to improve organoleptic properties, reduce toxic substances and anti-nutritional factors, and enhances the product's acceptability (Simatende et al. 2015). Originally, the production of fermented beverages was done to boost the shelf-life of perishable raw materials of agricultural and animal origin. Currently, the widely accepted concept of fermentation is the use of microorganisms and their enzymes for the production of fermented products through acidification, alcoholization, and proteolysis (Ashaolu 2019; De Roos and De Vuyst 2018).

During traditional fermentation of beverages, several groups of microorganisms are known to involve in the fermentation processes. While some microbes initiate the fermentation process, others dominate the fermentation process in succession until few strains finally takeover the remaining fermentation phases toward the end of fermentation (Kosisochukwu et al. 2020; Mulaw and Tesfaye 2017; Tsafakidou et al. 2020). Hence, certain microorganisms initiate the fermentation and dominate for a specific period; subsequently, the number declines due to the accumulation of different metabolites and inhibitory substances produced as by-products. In this way, the fermenting microorganisms provide an appropriate and conducive environment to less sensitive species while it inhibits the sensitive pathogens through the production of inhibitory factors (Chaves-Lopez et al. 2020).

In the early stage of fermentation, aerobic mesophiles such as staphylococci, micrococci, members of Enterobacteriaceae, and *Bacillus* dominate fermenting beverages. In the course of fermentation, however, the most common fermenting microorganisms such as *Leuconostoc*, *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Micrococcus*, *Saccharomyces cerevisiae*, *Kluyvermyces bulgaricus*, and *Debaromyces phaffi* dominate fermentation of

African indigenous beverages (Bahiru et al. 2006; Jespersen 2003; Ketema et al. 1998).

The dominant microorganisms at the end of fermentation of beverages are usually lactic acid bacteria and yeast, which resist the changes in the physicochemical properties of beverages, thus, persisting throughout fermentation (Nemo and Bacha 2020). Both LAB and yeast produce metabolites such as lactic acid, propionic acid, acetic acid, ethanol, and hydrogen peroxide, as well as other secondary metabolites/antimicrobial compounds/which are strong inhibitory substances for microbial growth (Siedler et al. 2019). These metabolites are potentially effective in controlling microbes including fungal growth (Adebisi et al. 2019). Due to their physicochemical characteristics, most of the high alcoholic beverages are considered microbiologically safe products, as the vegetative cells of pathogens cannot survive the high alcohol content, low pH, high carbon dioxide, low oxygen, and secondary metabolites (Kim et al. 2014). Pathogens such as *E. coli*, *S. typhimurium*, and *L. monocytogenes* survive and grow rapidly at low alcoholic beverages while 5% ethanol inhibits the growth and survival of the pathogens (Menz et al. 2010).

In Ethiopia, there are many traditional fermented beverages such as *tej*, *borde*, *tella*, *shameta*, *buquri*, *korefe*, *keribo*, *siljo*, and *grawa* (Lemi 2020; Nemo and Bacha 2020). Comprehensive data on the microbial dynamic and pattern of growth of potentially pathogenic organism in cases of contamination of the ready-to-consume beverages are scarce. Therefore, the present study aims to assess the microbial succession in the course of fermentation of *borde*, *tej*, and the newly described traditionally fermented beverage (*grawa*) and to evaluate the growth potential of selected pathogens in three of the fermented beverages.

## Materials and methods

### Description of the study area

The study was conducted in Jimma town located 353 km southwest of Addis Ababa, the capital city of Ethiopia. It is located at a latitude of about 7° 13 to 8° 56 N and longitudes of about 35° 52 to 37° 37E, an altitude ranging between 1720 to 2110 m above sea level, a mean annual rainfall of 800 to 2500 mm, and annual temperature ranging from 7 to 30 °C. The town is typically known for vending various traditional fermented beverages and foods of plants and animal sources (Nemo et al. 2017; Nemo and Bacha 2020).

### Sample collection and laboratory-scale preparation

The raw materials used for the preparation of the three beverages were purchased from open markets of Jimma town (for *borde* and *tej*), and Mugi town of Anfilo District located in Qellem Wollega Zone, for *grawa*. All raw

materials were transported to the Research and Post-graduate Laboratory, Department of Biology, College of Natural Sciences, Jimma University and used for laboratory-scale fermentation of the three traditionally fermented beverages for the determination of microbial succession during fermentation, and challenge testing of pathogens in the final products. Laboratory-scale preparation of the beverages was done by local brewers who had experience in preparing the selected beverages. The preparation protocol simulated the traditional technique followed by the local producers, and aseptic techniques were employed during preparation to avoid cross-contamination. The detailed flow charts of the traditional preparation techniques of the three beverages are as given below (Figs. 1, 2, 3).

#### Preparation of *borde*

*Borde* is prepared mainly from maize (*Zea mays*), barley (*Hordeum vulgare*), and malt. In this study, *borde* preparation did not use any starter culture to speed up the fermentation process either from previous fermentation (black slopes) or commercial starter culture (Fig. 1).

#### Preparation of *tej*

*Tej*, also called honey wine, is a home-processed beverage prepared from honey, water, malt, and chopped stems of hop (*Rhamnus prenoides*). In this study, the preparation of *tej* followed the simulated procedure of *tej* making in Jimma town (Fig. 2).

#### Preparation of *grawa*

*Grawa* is a fermented beverage prepared from specific honey made using flowers of *Vernonia amygdalina* and water from Anfilo District. *Grawa* from previous fermentation (back slope) has been used as a starter culture and the whole fermentation processes takes about 72 h (Fig. 3).

#### Microbial dynamics

Microbial successions in *grawa*, *borde*, and *tej* samples were assessed by microbiological enumeration during the 24 h fermentation period for *borde*, 72 h for *grawa*, and 144 h for *tej*. Samples were drawn at every 6 h gaps for *borde* and 12 h for *grawa* and *tej*. Accordingly, 10 ml of each sample was suspended in 90 ml of peptone water and homogenized in a flask for 10 min using a homogenizer (Edmund Buhler GmbH, Germany) at 100 rpm. Then, 1 ml of each sample was homogenized in 9 ml peptone water and serially diluted up to  $10^{-7}$ . Finally, 0.1 ml of aliquot was spread plated on plate count agar (PCA) for aerobic mesophilic bacteria and aerobic spore-forming bacteria (after heating the sample for 10 min at 80 °C for a count of the later), mannitol salt agar

(MSA) for staphylococci, violet red bile glucose agar (VRBGA) for Enterobacteriaceae, violet red bile agar (VRBA) for coliform, de Man Rogosa Sharpe (MRS) for lactic acid bacteria, and potato dextrose agar (PDA) supplemented with 200 mgL<sup>-1</sup> chloramphenicol for yeast and mold counts. All of the media were oxid and incubated at 32 °C for 48 h for bacteria and 28 °C for 2-5 days for yeast and mold. Moreover, the incubation condition for all microbes was aerobic except for lactic acid bacteria, which was incubated anaerobically using an anaerobic Jar (Hitech e-601, China).

#### Physicochemical analysis during beverage fermentation

##### pH

pH was measured using a digital portal pH meter (pH-013, China) after homogenizing 5 ml of *borde* sample in 20 ml of distilled water followed by pipetting 10 ml of the homogenized sample into a beaker (Kebede 2007). For *grawa* and *Tej* samples, 10 ml each was poured into a beaker and the electrode of the pH meter was dipped into the sample before recording the reading.

##### Titrateable acidity (TA)

The TA of *borde* was determined by homogenizing 5 ml of *borde* sample in 20 ml distilled water and filtered through Whatman no. 1 filter paper; then 9 ml of the homogenate was pipetted into a beaker (Antony and Chandra 1997). However, for *grawa* and *tej* samples, 9 ml of each sample was directly pipetted into a separate beaker. For all samples, 3 to 5 drops of 1 g/100 ml phenolphthalein indicator were added and titrated with 0.1 mol/L NaOH solution until a faint pink color persisted. The result was calculated using the following formula:

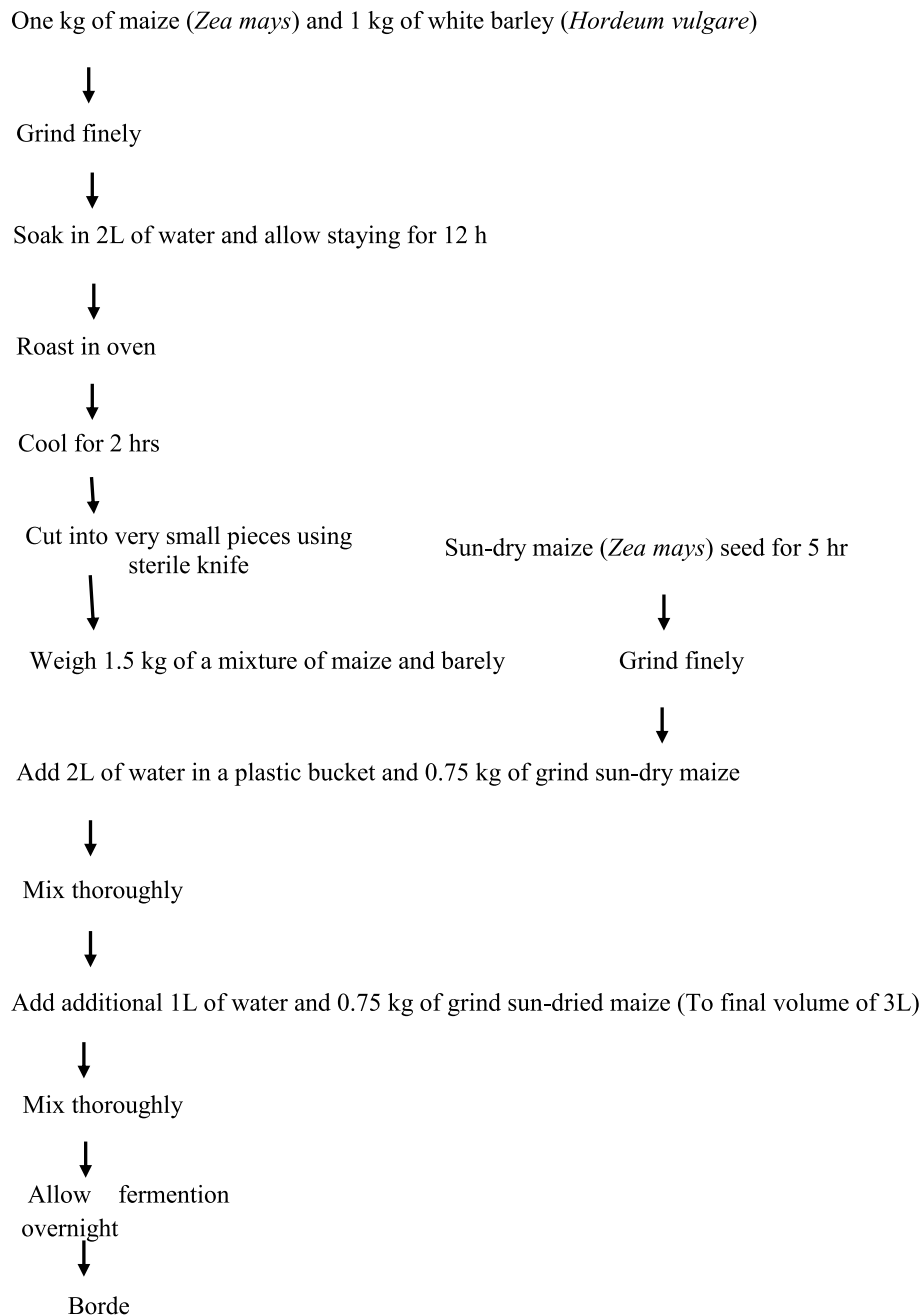
$$\text{Lactic acid (g/100ml)} = \frac{\text{Amount of NaOH titrated} \times \text{mol/L of NaOH} \times 9}{\text{The volume of sample (ml)}}$$

##### Moisture and total solid content

Moisture content was determined by oven drying (105 °C) of 10 ml of the *borde* samples until a constant weight is persisted. Then, the moisture content was calculated by subtracting the weight of the *borde* sample after drying *borde* sample + empty crucible to constant weight divided by *borde* sample + empty crucible minus empty crucible times 100 (AOAC, 1990). The total solid content was determined by subtracting the moisture content from 100.

##### Growth potential of pathogens in selected beverages

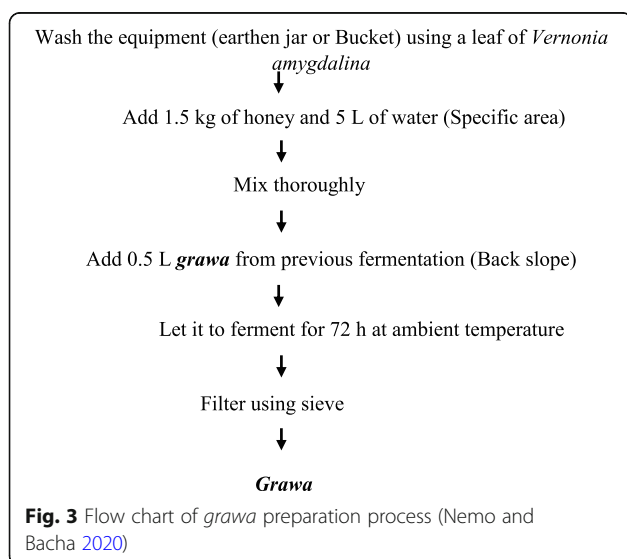
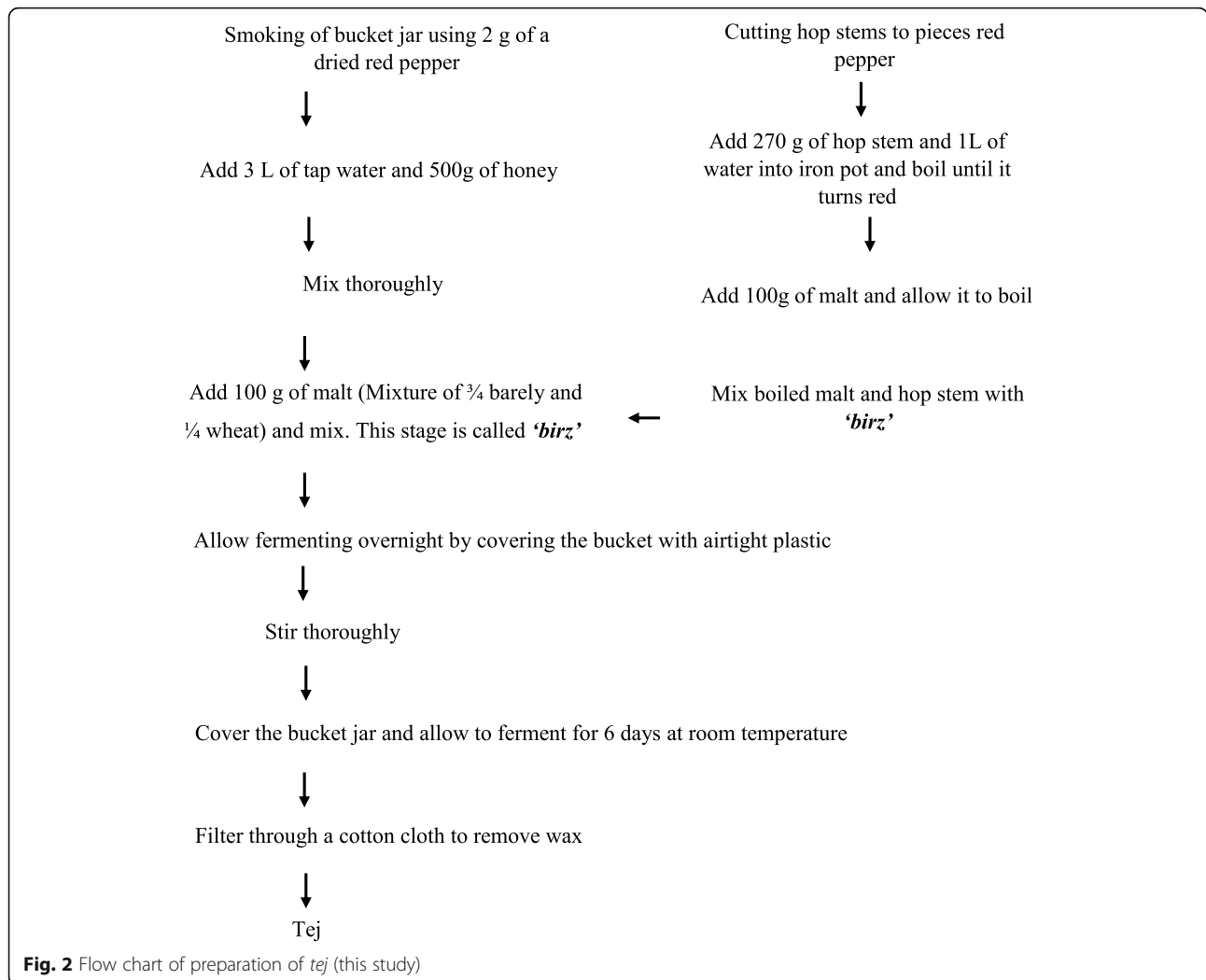
The selected beverage samples were separately challenged with reference strains including *Escherichia coli* (ATCC®25922™), *Salmonella enterica* subsp.



**Fig. 1** Flow chart of preparation of *borde* (this study)

*enterica* serovar Typhimurium (ATCC<sup>®</sup>13311<sup>™</sup>), *Staphylococcus aureus* subsp. *aureus* (ATCC<sup>®</sup> 25923<sup>™</sup>), *Listeria monocytogenes* (ATCC<sup>®</sup>19115<sup>™</sup>), and *Candida albicans* (ATCC<sup>®</sup>14053<sup>™</sup>) which were obtained from Ethiopian Public Health Institute (EPHI). Accordingly, 200 ml of each beverage was separately steamed at 80 °C for 10 min to kill any vegetative cells that might be present in the beverages. Then, 100 ml of each beverage was challenged separately with a 1 ml overnight culture of the test strains to bring the final

inoculum level to 10<sup>3</sup>-10<sup>4</sup> CFU/ml. Then, 10 ml of each beverage was homogenized in 90 ml of buffered peptone water and 0.1 ml of appropriate dilution was spread plated on eosin methylene blue agar for *E. coli*, xylose lysine deoxycholate agar for *S. typhimurium*, mannitol salt agar for *S. aureus*, Listeria selective agar for *L. monocytogenes*, and Sabouraud chloramphenicol agar for *C. albicans*. A portion of the beverage was further sampled aseptically at 6 h intervals from 0 to 24 h (Shamebo et al. 2016).



### Data analysis

The colonies counted from replicate plates were calculated in CFU/ml and later converted to log CFU/ml. The average mean counts during microbial succession, challenge testing, and data of physicochemical parameters were tested using one-way ANOVA analysis: post-hoc multiple comparisons, Tukey method, and a significant difference were considered at  $P < 0.05$  using SPSS version 26.

### Results

The three Ethiopian traditional fermented beverages (*tej*, *grawa*, and *borde*) are different in composition of ingredients used for their preparation as well as their uses. Both *grawa* and *tej* are yellow colored, had sweet taste, and used to quench thirsty and have medicinal values as used traditionally. On the other hand, *borde* is an opaque, effervescent, whitish-gray to brown colored beverage, commonly used to feed lactating mothers, besides its use as meal replacement

among segment of the population who cannot afford full meal (Fig. 4).

#### Microbial dynamics

At the beginning of *borde* fermentation (0 h), aerobic mesophilic bacteria (AMB), staphylococci, and Enterobacteriaceae initiated the fermentation process and gradually started to dominate with counts exceeding  $> 5 \log$  CFU/ml. The AMB was continued to dominant until 6 h of fermentation with a significant difference ( $p < 0.05$ ) in count from 0 h. At 12 h, and thereafter until the end of fermentation, LAB and yeast dominated the fermentation and reached the maximum counts of  $7.33 \pm 0.07$  and  $6.91 \pm 0.04 \log$  CFU/ml, respectively. Similarly, in *grawa* beverage, at 0 h the count of AMB was higher ( $5.06 \pm 0.02 \log$  CFU/ml) than other microorganisms. The dominance of AMB was continued until 24 h, and after 24 h, the yeast and LAB dominated the fermentation process until the end of fermentation (72 h) and reached the maximum counts of ( $7.88 \pm 0.02$  and  $7.64 \pm 0.04 \log$  CFU/ml, respectively). Likewise, in *tej* fermentation, the counts of AMB until 36 h, staphylococci, and ASFB until 24 h increased by more than 1 log CFU/ml and later dropped down with a significant difference ( $P < 0.05$ ). However, the count of yeast was increased by greater than 5 log ( $4.16 \pm 0.04$  to  $9.41 \pm 0.06 \log$  CFU/ml) and LAB was increased by nearly 5 log ( $4.01 \pm 0.03$  to  $8.88 \pm 0.01 \log$  CFU/ml) with a significant difference in counts between fermentation hours analyzed from the beginning (0 h) to the end of fermentation time (144 h). In all three beverages, the counts of molds from the beginning of fermentation, and counts of coliform, and Enterobacteriaceae after a certain time were recorded as less than 2 log CFU/ml (Table 1).

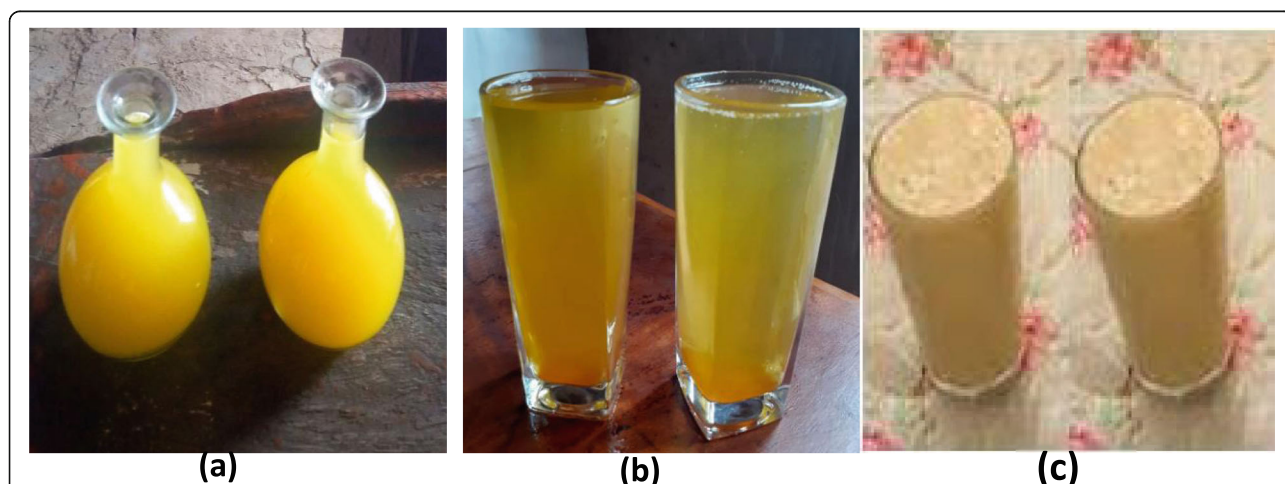
In general, the counts of AMB showed a significant difference until 12 h for *borde* and *grawa* samples, and 24 h for *tej* sample. However, the counts of staphylococci showed no significant difference until 2 h for *grawa* and 6 h for *borde*. The counts of LAB and yeast showed significant difference in almost all the three beverage samples (Table 1).

#### Physico-chemical changes during beverage fermentations

During microbial succession, the pH values of *borde* and *grawa* samples were dropped from  $4.58 \pm 0.03$  to  $4.22 \pm 0.01$  and  $4.18 \pm 0.10$  to  $3.62 \pm 0.02$ , respectively, from the beginning to the end of fermentation time. However, the pH of *tej* increased for the first 24 h and dropped thereafter to a minimum of  $3.81 \pm 0.02$  at 144 h. The titratable acidity and moisture content of the beverages were increased as fermentation time increased, while the total solid content was decreased. On the other hand, in *borde* and *grawa* samples, there was no significant difference ( $P > 0.05$ ) in changes in pH from 0 to 6 h and 0 to 12 h, respectively. Moreover, there was also no significant difference ( $p < 0.05$ ) in titratable acidity of *borde* from 0 to 6 h of fermentation, and *tej* from 0 to 24 h of fermentation. However, there was a significant difference in titratable acidity in most fermentation times (Table 2).

#### Growth potential of pathogens in selected beverages

In a microbial challenge test of *borde* sample, the counts of all pathogens were increased significantly until the end of the challenge test. However, the rate of growth varied whereby the counts of *E. coli* ( $3.15 \pm 0.03$  to  $5.82 \pm 0.08 \log$  CFU/ml) and *S. aureus* ( $3.72 \pm 0.05$  to  $6.05 \log$  CFU/ml) increased by more than 2.3 log CFU/ml



**Fig. 4** Pictures of Ethiopian traditional fermented beverages: *tej* (a), *grawa* (b), and *borde* (c). *Tej* is prepared from honey, water, malt, and chopped stems of hop (*Rhamnus prenoides*), while *grawa* is made from only honey and water of Qellem Wollega Zone, Anfilo District. *Borde* is usually made from maize (*Zea mays*) and wheat (*Triticum* spp.)

**Table 1** Microbial dynamics (mean  $\pm$  SD) during fermentation of selected Ethiopian traditional fermented beverages

Beverages	Fermentation time (h)	Mean microbial count (Log CFU/ml)						
		AMB	Staphylococci	Enterobacteriaceae	Coliform	ASFB	LAB	Yeast
<i>Borde</i>	0	6.42 $\pm$ 0.10 <sup>a</sup>	5.44 $\pm$ 0.08 <sup>a</sup>	5.40 $\pm$ 0.11 <sup>a</sup>	3.35 $\pm$ 0.07 <sup>a</sup>	4.45 $\pm$ 0.06 <sup>a</sup>	4.75 $\pm$ 0.04 <sup>d</sup>	4.12 $\pm$ 0.16 <sup>c</sup>
	6	6.15 $\pm$ 0.05 <sup>b</sup>	5.29 $\pm$ 0.06 <sup>a</sup>	4.17 $\pm$ 0.07 <sup>b</sup>	2.60 $\pm$ 0.11 <sup>b</sup>	4.26 $\pm$ 0.04 <sup>a</sup>	5.34 $\pm$ 0.01 <sup>c</sup>	4.45 $\pm$ 0.30 <sup>c</sup>
	12	5.20 $\pm$ 0.02 <sup>c</sup>	4.87 $\pm$ 0.01 <sup>b</sup>	3.85 $\pm$ 0.09 <sup>c</sup>	< 2	4.04 $\pm$ 0.06 <sup>b</sup>	5.55 $\pm$ 0.14 <sup>c</sup>	5.22 $\pm$ 0.09 <sup>b</sup>
	18	5.10 $\pm$ 0.01 <sup>c</sup>	4.15 $\pm$ 0.05 <sup>c</sup>	< 2	< 2	3.98 $\pm$ 0.05 <sup>bc</sup>	6.09 $\pm$ 0.04 <sup>b</sup>	5.86 $\pm$ 0.06 <sup>b</sup>
	24	4.99 $\pm$ 0.06 <sup>c</sup>	3.85 $\pm$ 0.09 <sup>d</sup>	< 2	< 2	3.77 $\pm$ 0.11 <sup>c</sup>	7.33 $\pm$ 0.07 <sup>a</sup>	6.91 $\pm$ 0.04 <sup>a</sup>
<i>Grawa</i>	0	5.06 $\pm$ 0.02 <sup>c</sup>	4.91 $\pm$ 0.06 <sup>a</sup>	4.29 $\pm$ 0.06 <sup>c</sup>	4.13 $\pm$ 0.03 <sup>a</sup>	3.89 $\pm$ 0.06 <sup>b</sup>	4.63 $\pm$ 0.03 <sup>e</sup>	4.73 $\pm$ 0.02 <sup>e</sup>
	12	6.30 $\pm$ 0.12 <sup>a</sup>	5.14 $\pm$ 0.01 <sup>a</sup>	5.16 $\pm$ 0.04 <sup>a</sup>	4.11 $\pm$ 0.03 <sup>a</sup>	4.56 $\pm$ 0.14 <sup>a</sup>	5.70 $\pm$ 0.02 <sup>d</sup>	5.40 $\pm$ 0.08 <sup>d</sup>
	24	6.41 $\pm$ 0.04 <sup>a</sup>	5.08 $\pm$ 0.09 <sup>a</sup>	4.73 $\pm$ 0.06 <sup>b</sup>	4.08 $\pm$ 0.06 <sup>a</sup>	4.51 $\pm$ 0.01 <sup>a</sup>	6.26 $\pm$ 0.09 <sup>c</sup>	6.06 $\pm$ 0.08 <sup>c</sup>
	36	5.54 $\pm$ 0.05 <sup>b</sup>	4.37 $\pm$ 0.04 <sup>b</sup>	3.61 $\pm$ 0.08 <sup>d</sup>	3.03 $\pm$ 0.02 <sup>b</sup>	4.49 $\pm$ 0.01 <sup>a</sup>	6.49 $\pm$ 0.03 <sup>b</sup>	6.39 $\pm$ 0.06 <sup>bc</sup>
	48	4.73 $\pm$ 0.14 <sup>d</sup>	3.95 $\pm$ 0.14 <sup>c</sup>	2.80 $\pm$ 0.02 <sup>e</sup>	2.21 $\pm$ 0.13 <sup>c</sup>	3.39 $\pm$ 0.08 <sup>c</sup>	6.45 $\pm$ 0.01 <sup>bc</sup>	6.67 $\pm$ 0.16 <sup>b</sup>
	60	3.82 $\pm$ 0.05 <sup>e</sup>	3.62 $\pm$ 0.07 <sup>d</sup>	< 2	< 2	3.06 $\pm$ 0.08 <sup>d</sup>	7.46 $\pm$ 0.09 <sup>a</sup>	7.71 $\pm$ 0.09 <sup>a</sup>
	72	3.39 $\pm$ 0.02 <sup>f</sup>	2.77 $\pm$ 0.01 <sup>e</sup>	< 2	< 2	2.58 $\pm$ 0.03 <sup>e</sup>	7.64 $\pm$ 0.04 <sup>a</sup>	7.88 $\pm$ 0.02 <sup>a</sup>
<i>Tej</i>	0	5.37 $\pm$ 0.04 <sup>c</sup>	3.90 $\pm$ 0.02 <sup>d</sup>	4.34 $\pm$ 0.07 <sup>c</sup>	3.23 $\pm$ 0.02 <sup>c</sup>	4.13 $\pm$ 0.02 <sup>c</sup>	4.01 $\pm$ 0.03 <sup>k</sup>	4.16 $\pm$ 0.04 <sup>i</sup>
	12	5.94 $\pm$ 0.04 <sup>b</sup>	4.73 $\pm$ 0.01 <sup>b</sup>	4.70 $\pm$ 0.06 <sup>b</sup>	3.55 $\pm$ 0.01 <sup>a</sup>	4.25 $\pm$ 0.01 <sup>bc</sup>	4.83 $\pm$ 0.06 <sup>j</sup>	4.97 $\pm$ 0.04 <sup>h</sup>
	24	6.58 $\pm$ 0.02 <sup>a</sup>	5.63 $\pm$ 0.11 <sup>a</sup>	5.17 $\pm$ 0.07 <sup>a</sup>	3.51 $\pm$ 0.08 <sup>ab</sup>	5.42 $\pm$ 0.01 <sup>a</sup>	5.53 $\pm$ 0.13 <sup>i</sup>	5.39 $\pm$ 0.07 <sup>g</sup>
	36	6.62 $\pm$ 0.01 <sup>a</sup>	4.41 $\pm$ 0.03 <sup>c</sup>	4.26 $\pm$ 0.04 <sup>c</sup>	3.38 $\pm$ 0.07 <sup>b</sup>	4.36 $\pm$ 0.03 <sup>b</sup>	5.94 $\pm$ 0.03 <sup>h</sup>	6.62 $\pm$ 0.07 <sup>f</sup>
	48	6.36 $\pm$ 0.16 <sup>a</sup>	4.31 $\pm$ 0.02 <sup>c</sup>	3.82 $\pm$ 0.06 <sup>d</sup>	3.15 $\pm$ 0.04 <sup>c</sup>	4.17 $\pm$ 0.10 <sup>c</sup>	6.60 $\pm$ 0.08 <sup>g</sup>	6.77 $\pm$ 0.01 <sup>f</sup>
	60	5.41 $\pm$ 0.09 <sup>c</sup>	4.05 $\pm$ 0.08 <sup>d</sup>	3.28 $\pm$ 0.08 <sup>e</sup>	2.83 $\pm$ 0.03 <sup>d</sup>	3.83 $\pm$ 0.06 <sup>d</sup>	6.65 $\pm$ 0.04 <sup>g</sup>	6.84 $\pm$ 0.06 <sup>f</sup>
	72	5.29 $\pm$ 0.08 <sup>c</sup>	3.93 $\pm$ 0.02 <sup>d</sup>	3.13 $\pm$ 0.01 <sup>ef</sup>	< 2	3.21 $\pm$ 0.01 <sup>e</sup>	7.10 $\pm$ 0.03 <sup>f</sup>	7.40 $\pm$ 0.05 <sup>e</sup>
	84	4.60 $\pm$ 0.10 <sup>d</sup>	3.22 $\pm$ 0.02 <sup>e</sup>	3.01 $\pm$ 0.04 <sup>f</sup>	< 2	3.14 $\pm$ 0.01 <sup>e</sup>	7.51 $\pm$ 0.07 <sup>e</sup>	7.53 $\pm$ 0.04 <sup>e</sup>
	96	4.28 $\pm$ 0.21 <sup>d</sup>	2.77 $\pm$ 0.01 <sup>f</sup>	2.55 $\pm$ 0.17 <sup>g</sup>	< 2	2.83 $\pm$ 0.05 <sup>f</sup>	7.84 $\pm$ 0.07 <sup>d</sup>	8.43 $\pm$ 0.06 <sup>d</sup>
	108	3.64 $\pm$ 0.04 <sup>e</sup>	2.55 $\pm$ 0.01 <sup>g</sup>	< 2	< 2	2.69 $\pm$ 0.07 <sup>fg</sup>	8.15 $\pm$ 0.06 <sup>c</sup>	8.61 $\pm$ 0.07 <sup>cd</sup>
	120	3.52 $\pm$ 0.04 <sup>e</sup>	2.34 $\pm$ 0.02 <sup>h</sup>	< 2	< 2	2.51 $\pm$ 0.07 <sup>gh</sup>	8.41 $\pm$ 0.08 <sup>bc</sup>	8.76 $\pm$ 0.11 <sup>c</sup>
	132	3.45 $\pm$ 0.03 <sup>e</sup>	2.13 $\pm$ 0.01 <sup>i</sup>	< 2	< 2	2.34 $\pm$ 0.01 <sup>hi</sup>	8.62 $\pm$ 0.08 <sup>ab</sup>	9.01 $\pm$ 0.04 <sup>b</sup>
	144	3.42 $\pm$ 0.01 <sup>e</sup>	2.07 $\pm$ 0.06 <sup>i</sup>	< 2	< 2	2.22 $\pm$ 0.02 <sup>i</sup>	8.88 $\pm$ 0.01 <sup>a</sup>	9.41 $\pm$ 0.06 <sup>a</sup>

AMB aerobic mesophilic bacteria, ASFB aerobic spore forming bacteria, LAB lactic acid bacteria

Evaluation of the microbial dynamics was conducted following the traditional fermentation techniques and their respective duration of fermentation: 24 h for *borde* (sampling every 6 h), 72 h of fermentation for *grawa*, and 144 h for *tej* (sampling every 12 h). The patterns of microbial dynamics of the three beverages were presented with mean microbial counts (mean  $\pm$  SD of Log CFU/ml). The statistical analysis was determined by one-way ANOVA with Tukey post hoc test. Different letters in superscript along a column indicate a significant difference ( $p < 0.05$ ), the same letters indicate no significant difference ( $p > 0.05$ )

while *S. typhimurium* (3.48  $\pm$  0.04 to 5.44  $\pm$  0.01 log CFU/ml) and *C. albicans* (3.57  $\pm$  0.07 to 5.09  $\pm$  0.09 log CFU/ml) increased by less than 2 log CFU/ml (Fig. 5A). In the *grawa* sample, fast reduction (0.52 log) of *L. monocytogenes* was observed from the initial 0 h (3.90  $\pm$  0.02 CFU/ml) to 6 h (3.38  $\pm$  0.03 log CFU/ml) with a significant difference ( $p < 0.05$ ) between counts, while *S. typhimurium* and *E. coli* were reduced by 0.1 log with no significant difference ( $p > 0.05$ ) between counts of different sampling hours. At the end of microbial challenge testing, *E. coli*, *S. typhimurium*, *S. aureus*, and *L. monocytogenes* were reduced by > 1 log unit. However, *C. albicans* was reduced by 0.7 log (Fig. 5B). Moreover, in *tej* sample, *C. albicans* was reduced to a lesser extent (0.88 CFU/ml) when compared to other pathogens. However, *L. monocytogenes* were highly reduced (2.2 CFU/ml) with a significant difference (Fig. 5C) among

counts. The pH in all samples drawn during the challenge test did show no remarkable difference.

## Discussion

During the fermentation of *borde*, early hours of fermentation were dominated by AMB, staphylococci, Enterobacteriaceae, and ASFB. However, at the end of fermentation LAB and yeast dominated the fermentation and reached the maximum count of 7.33  $\pm$  0.07 and 6.91  $\pm$  0.04, respectively. In line with the present study, Kosisochukwu et al. (2020) reported that microbes such as *Streptococcus*, *Bacillus*, and *Corynebacterium* started the fermentation which were later dominated by LAB and yeast during *ogi* fermentation (usually produced from the fermentation of maize or other cereals). Similarly, Ketema et al. (1998) reported that the onset of fermentation of *borde* was dominated by staphylococci, *Bacillus*, and members of Enterobacteriaceae. In a related

**Table 2** Some physicochemical characteristics (mean  $\pm$  SD) during laboratory-scale fermentation of selected beverages

Sample	Fermentation time (h)	pH	Titrateable acid	Moisture content (g/100 ml)	Total solid content (g/100 ml)
<i>Borde</i>	0	4.58 $\pm$ 0.03 <sup>a</sup>	0.17 $\pm$ 0.02 <sup>d</sup>	74.17 $\pm$ 0.75 <sup>d</sup>	25.83 $\pm$ 0.75 <sup>a</sup>
	6	4.55 $\pm$ 0.02 <sup>a</sup>	0.21 $\pm$ 0.01 <sup>d</sup>	76.65 $\pm$ 0.48 <sup>c</sup>	23.35 $\pm$ 0.48 <sup>b</sup>
	12	4.31 $\pm$ 0.01 <sup>b</sup>	0.25 $\pm$ 0.02 <sup>c</sup>	77.59 $\pm$ 0.04 <sup>bc</sup>	22.41 $\pm$ 0.04 <sup>bc</sup>
	18	4.27 $\pm$ 0.02 <sup>bc</sup>	0.32 $\pm$ 0.01 <sup>b</sup>	78.48 $\pm$ 0.36 <sup>b</sup>	22.41 $\pm$ 0.36 <sup>c</sup>
	24	4.22 $\pm$ 0.01 <sup>c</sup>	0.40 $\pm$ 0.02 <sup>a</sup>	82.79 $\pm$ 0.83 <sup>a</sup>	21.52 $\pm$ 0.83 <sup>d</sup>
<i>Grawa</i>	0	4.18 $\pm$ 0.10 <sup>a</sup>	0.19 $\pm$ 0.02 <sup>f</sup>	85.57 $\pm$ 0.08 <sup>d</sup>	14.43 $\pm$ 0.08 <sup>a</sup>
	12	4.16 $\pm$ 0.06 <sup>a</sup>	0.22 $\pm$ 0.01 <sup>ef</sup>	85.71 $\pm$ 0.03 <sup>d</sup>	14.29 $\pm$ 0.03 <sup>a</sup>
	24	4.11 $\pm$ 0.08 <sup>ab</sup>	0.25 $\pm$ 0.02 <sup>de</sup>	85.92 $\pm$ 0.20 <sup>cd</sup>	14.08 $\pm$ 0.20 <sup>ab</sup>
	36	3.96 $\pm$ 0.03 <sup>bc</sup>	0.29 $\pm$ 0.01 <sup>cd</sup>	85.94 $\pm$ 0.21 <sup>cd</sup>	14.06 $\pm$ 0.21 <sup>ab</sup>
	48	3.90 $\pm$ 0.02 <sup>cd</sup>	0.33 $\pm$ 0.02 <sup>c</sup>	86.32 $\pm$ 0.21 <sup>c</sup>	13.68 $\pm$ 0.21 <sup>b</sup>
	60	3.76 $\pm$ 0.02 <sup>de</sup>	0.57 $\pm$ 0.03 <sup>b</sup>	88.15 $\pm$ 0.32 <sup>b</sup>	11.85 $\pm$ 0.32 <sup>c</sup>
	72	3.62 $\pm$ 0.02 <sup>e</sup>	0.83 $\pm$ 0.08 <sup>a</sup>	89.96 $\pm$ 0.28 <sup>a</sup>	10.04 $\pm$ 0.28 <sup>d</sup>
<i>Tej</i>	0	5.26 $\pm$ 0.01 <sup>c</sup>	0.13 $\pm$ 0.01 <sup>h</sup>	74.28 $\pm$ 0.58 <sup>k</sup>	25.72 $\pm$ 0.58 <sup>a</sup>
	12	5.36 $\pm$ 0.02 <sup>b</sup>	0.15 $\pm$ 0.01 <sup>h</sup>	75.36 $\pm$ 0.23 <sup>k</sup>	24.64 $\pm$ 0.23 <sup>a</sup>
	24	5.50 $\pm$ 0.03 <sup>a</sup>	0.12 $\pm$ 0.01 <sup>h</sup>	76.83 $\pm$ 0.24 <sup>j</sup>	23.17 $\pm$ 0.24 <sup>b</sup>
	36	4.85 $\pm$ 0.01 <sup>d</sup>	0.23 $\pm$ 0.01 <sup>g</sup>	77.11 $\pm$ 0.56 <sup>ij</sup>	22.89 $\pm$ 0.56 <sup>bc</sup>
	48	4.58 $\pm$ 0.02 <sup>e</sup>	0.27 $\pm$ 0.03 <sup>g</sup>	78.01 $\pm$ 0.21 <sup>hi</sup>	21.90 $\pm$ 0.21 <sup>cd</sup>
	60	4.27 $\pm$ 0.03 <sup>f</sup>	0.34 $\pm$ 0.04 <sup>f</sup>	78.82 $\pm$ 0.20 <sup>gh</sup>	21.18 $\pm$ 0.20 <sup>de</sup>
	72	4.19 $\pm$ 0.01 <sup>g</sup>	0.38 $\pm$ 0.02 <sup>ef</sup>	79.51 $\pm$ 0.16 <sup>fg</sup>	20.49 $\pm$ 0.16 <sup>ef</sup>
	84	4.14 $\pm$ 0.02 <sup>gh</sup>	0.39 $\pm$ 0.04 <sup>ef</sup>	80.56 $\pm$ 0.59 <sup>ef</sup>	19.44 $\pm$ 0.59 <sup>fg</sup>
	96	4.11 $\pm$ 0.01 <sup>hi</sup>	0.42 $\pm$ 0.03 <sup>de</sup>	81.44 $\pm$ 0.29 <sup>e</sup>	18.56 $\pm$ 0.29 <sup>g</sup>
	108	4.05 $\pm$ 0.04 <sup>i</sup>	0.46 $\pm$ 0.01 <sup>cd</sup>	83.18 $\pm$ 0.64 <sup>d</sup>	16.82 $\pm$ 0.64 <sup>h</sup>
	120	3.94 $\pm$ 0.05 <sup>j</sup>	0.49 $\pm$ 0.01 <sup>bc</sup>	84.93 $\pm$ 0.25 <sup>c</sup>	15.07 $\pm$ 0.25 <sup>i</sup>
	132	3.85 $\pm$ 0.01 <sup>k</sup>	0.54 $\pm$ 0.01 <sup>ab</sup>	86.89 $\pm$ 0.29 <sup>b</sup>	13.11 $\pm$ 0.29 <sup>j</sup>
	144	3.81 $\pm$ 0.02 <sup>k</sup>	0.58 $\pm$ 0.01 <sup>a</sup>	88.76 $\pm$ 0.22 <sup>a</sup>	11.24 $\pm$ 0.22 <sup>k</sup>

The physicochemical characteristics of the fermented beverages were assessed based on the beverages fermentation times: 24, 72, and 144 h for *borde*, *tej*, and *grawa*, respectively. The mean  $\pm$  standard is presented and the statistical analysis was determined by one-way ANOVA with Tukey post hoc test. Different letters in superscript along a column indicate a significant difference ( $p < 0.05$ ), the same letters indicate no significant difference ( $p > 0.05$ )

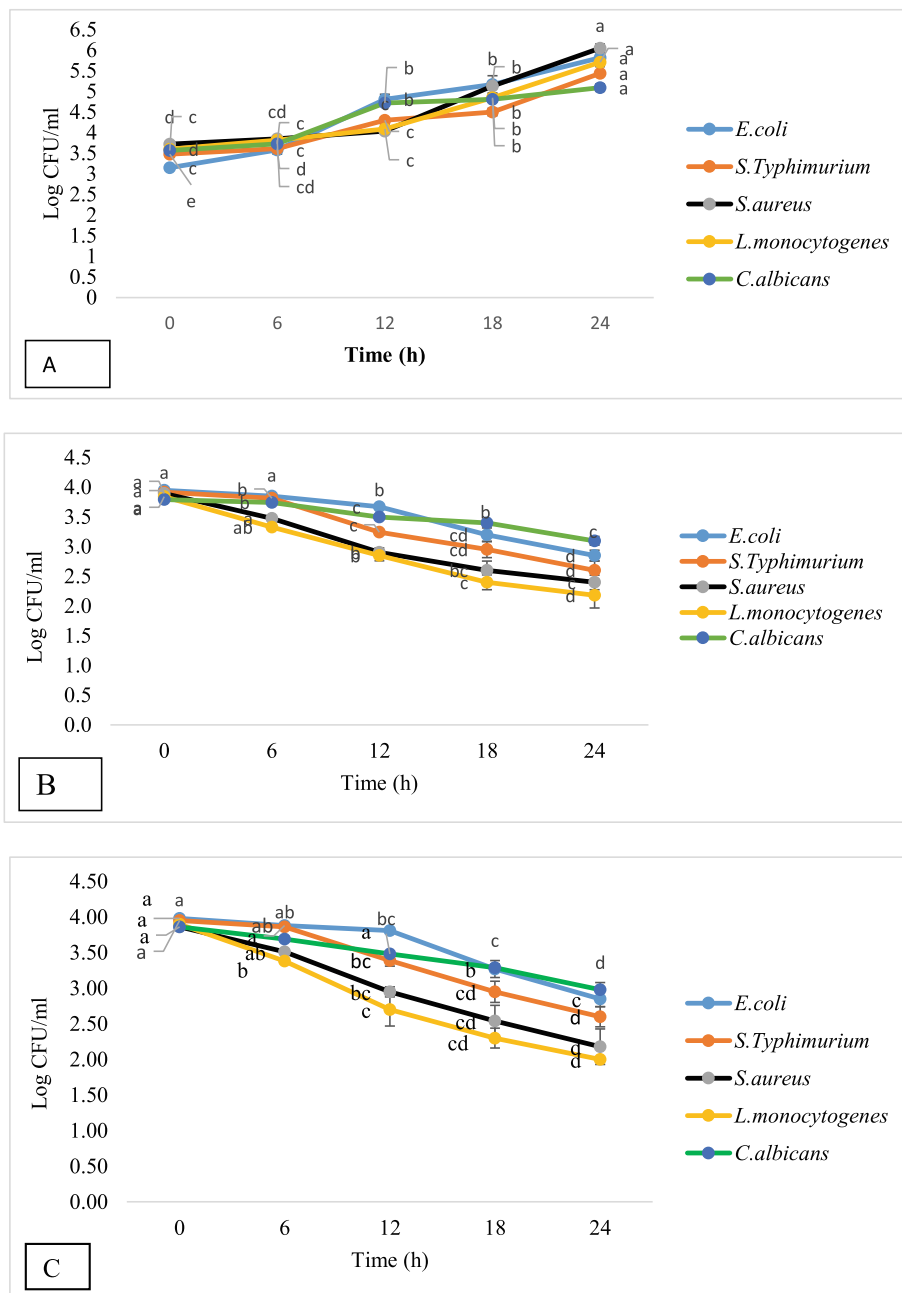
development, Nemo and Bacha (2020) reported maximum count of LAB and yeast at the level of  $6.87 \pm 0.67$  and  $6.56 \pm 0.81$  CFU/ml, respectively, from *borde* samples collected from Jimma and Danaba towns. The dominance of Enterobacteriaceae in early fermentation initiate the fermentation by utilizing simple sugars produced after the action of amylase from malt and amylolytic microorganisms and produce some basic vitamins used by fermentative microorganisms (LAB and yeast) for the production of organic acids, flavor compounds, peptides, ethanol, and aroma compounds (Ewuoso et al. 2020; Ketema et al. 1998). On the other hand, the presence of some aerobic spore-forming bacteria (*Bacillus* spp.) secrete a wide range of degradative enzymes, such as amylases and proteases, and thus playing an important role in sustaining the supply of simple sugars for fermentation processes to continue (Almeida et al. 2007).

At the end of the fermentation of *grawa*, the counts of yeast and LAB reached the maximum counts ( $7.88 \pm 0.02$  and  $7.64 \pm 0.04$  log CFU/ml, respectively). In higher

counts to the present study, Nemo and Bacha (2020) reported  $8.43 \pm 0.72$  log CFU/ml of yeast and  $8.13 \pm 0.67$  log CFU/ml of LAB from *grawa* beverage collected from Anfilo District of Qellem Wollega Zone, Southwest Ethiopia. The lower count of yeast and LAB in the present study could be due to environmental factors. Even though the production process followed was similar during *grawa* fermentation, environmental conditions such as temperature and moisture content could lead to vibrant differences in the kinetics and the product besides the microbial profile of the final product (Kirchmayr et al. 2017).

During *tej* fermentation, the counts of AMB, staphylococci, and ASFB were increased significantly ( $p < 0.05$ ) in the first 24 h and later dropped. However, the counts of yeast and LAB continuously increased until the end of fermentation and reached the maximum counts of  $9.41 \pm 0.06$  and  $8.88 \pm 0.01$  log CFU/ml, respectively. This report was much higher than the report by Nemo and





**Fig. 5** Growth potential of pathogens in *borde* (A), *grawa* (B), and *tej* (C) beverages. The growth potential of *E. coli*, *S. typhimurium*, *S. aureus*, *L. monocytogenes*, and *C. albicans* in the three beverages was assessed within 24 h by drawing samples at an interval of 6 h. The growth or no growth of these pathogens was determined having cultured the drawn samples on the pathogens' respective media, incubated at 37 °C, and counted viable cells which latter expressed in Log CFU/ml. The mean ± standard is presented and the statistical analysis was determined using one-way ANOVA with Tukey post hoc test. Different letters in superscript along a column indicate presence of significant difference ( $p < 0.05$ ), while the same letters indicate absence of significant difference ( $p > 0.05$ )

Bacha (2020), who reported  $6.31 \pm 0.63$  and  $6.09 \pm 0.53$  log CFU/ml from Jimma town *tej* vendors. And report by Bahiru et al. (2006) who reported yeast and lactic acid flora counts ( $< 7$  log CFU/ml) of *tej* from an indigenous Ethiopian honey wine. The increment of counts observed during the first 24 h dropped later due to pH

change (increased in the first 24 h and dropped later). Moreover, the observed higher count of yeast and LAB could likely be due to appropriate utilization of raw materials or ingredients (honey, wheat, and barley) without adulterating. Mostly, the local *tej* retailer uses sugar commercially produced in industries instead of using

honey as raw materials mainly because the honey price is getting expensive.

In *tej* fermentation, the presence highest counts of yeast and LAB co-existed was originated from the raw materials: honey, malt, and hops. In traditional fermented beverages, the source of microorganisms involved in the fermentation processes predominantly originates from microflora naturally present in the substrates and utensils/ equipment used for fermentation. During fermentation, microbial group coexisting together to enhance the fermentation processes by adapting to the changing intrinsic and extrinsic conditions caused by the physicochemical changes associated with microbial activity, duration of fermentation, temperature, and moisture content (Navarrete-bola 2012; Tamang et al. 2016).

In general, locally consumed beverages usually use rudimentary materials for preparation, and hence the microbial succession in due course of fermentation usually depends on the physicochemical characteristics of the fermenting matrix. Accordingly, the physicochemical properties of the selected beverages investigated in the current study showed a significant difference ( $p < 0.05$ ) in their physicochemical properties from the initial fermentation hours to the end of fermentation. Specifically, there was a reduction of pH in *tej* ( $5.26 \pm 0.01$  to  $3.81 \pm 0.02$ ), *borde* ( $4.58 \pm 0.03$  to  $4.22 \pm 0.01$ ), and *grawa* ( $4.18 \pm 0.10$  to  $3.62 \pm 0.02$ ) at the end of the fermentation while titratable acidity increased. The reduction in pH during fermentation time is accounted to change in an organic substrate to different end-products mainly organic acids esters and carbonyls by the action of the dominant microbes like LAB and yeast (Peyer et al. 2016). Titratable acidities increased significantly with days of fermentation in association with the accumulation of dominant metabolites such as lactic acid and acetic acid as opposed to a decline in pH (Liang et al. 2020).

In the present study, there was an increment of moisture contents (g/100 ml) in all samples; *borde* ( $74.17 \pm 0.75$  to  $82.79 \pm 0.83$ ), *grawa* ( $85.57 \pm 0.08$  to  $89.96 \pm 0.28$ ), and *tej* ( $74.28 \pm 0.58$  to  $88.76 \pm 0.22$ ). The highest moisture content (g/100 ml) at the end of fermentation was lower than the report of Nemo and Bacha (2020), who reported  $87.29 \pm 3.21$  in *borde*,  $95.84 \pm 1.10$  in *grawa*, and  $95.78 \pm 1.21$  in *tej*. The increase in the moisture content can be attributed to the addition of water to the substrate before fermentation (Ekundayo et al. 2013). The increment of moisture content with fermentation time is the consumption of dry matter and water production during aerobic and anaerobic catabolism by yeasts and lactic acid bacteria (Laetitia et al. 2005).

Microbiological challenge testing is a valuable approach to determine a food or beverage ability to

support the growth of the specific pathogens. In the present study, the growth of all reference pathogens in two beverages (*grawa* and *tej*) was gradually reduced and survived for only certain periods. The pH, alcoholic content, secondary metabolites of LAB, and yeast could be the main factors that are responsible for the reduction of pathogens inoculated for the beverages. The pH of *grawa* and *tej* in the present study was less than 4 until the end of the challenge test. Fermented alcoholic beverages contain valuable nutrients that have health benefits and are mostly acknowledged as microbiologically safe due to their high ethanol content greater than 4% and low pH (less than 4.5) (Jeon et al. 2015). According to the Nemo and Bacha (2020) report, *grawa* and *tej* had low pH ( $< 4$ ) and higher alcoholic content ( $> 4\%$ ). Occasionally, pathogens can survive extremely low pH ( $< 4.00$ ) for a certain time by pumping out of protons, production of ammonia, and proton-consuming through processes of decarboxylation of carboxyl groups of amino acids of proteins source reaction and the pH value 4.0-5.0 can support growth (Lund et al. 2014; Vivijs et al. 2016).

In this study, *E. coli* and *S. typhimurium* showed higher survival ability than *S. aureus* and *L. monocytogenes* in *grawa* and *tej* samples. Moreover, *C. albicans* were more resistant than all pathogens challenged. The better survival ability of Gram-negative bacteria than Gram-positive could be due to the cell-free supernatant of LAB and the ability to respond to stress. Cell-free supernatant of LAB has good antimicrobial properties against Gram-positive or closely related microorganisms than Gram-negative (Mora-Villalobos et al. 2020). *E. coli* is capable of producing stress response mechanisms that facilitate its adaptation to acid tolerance, which provides more resistance in acidic environments (Chauret 2011). *C. albicans* has an extraordinary capacity to alter extracellular pH, creating a neutral environment from either acidic or alkaline starting conditions, with changes in the pH units when glucose is repressible and requires exogenous amino acids (Vylkova 2017).

In the current study, all pathogens reach the infective dose in *borde* beverage. *Borde* supported the growth of pathogens due to low alcoholic content, less acidic (4.22) due to the short fermentation time of the beverages (an overnight fermentation), and has a good nutritional profile for the proliferation of microorganisms. Foodborne pathogens like *E. coli* O157:H7, *Staphylococcus aureus*, *Shigella flexneri*, and *Salmonella* spp. can survive and grow in fermenting *borde* (Tadesse et al. 2005). According to Nemo and Bacha (2020) report, *borde* has low alcoholic content, good nutritional value with relatively high pH that supports the growth of some undesired microorganisms.

## Conclusion

The selected beverages fermentation process was initiated by AMB, Enterobacteriaceae, staphylococci, and ASFB, and later due to the changes in physicochemical properties, LAB and yeast (the most fermentative and less affected by physicochemical changes) dominated the fermentation. The higher counts of LAB and yeast in *tej* were a potential source for industrial purposes: enzymes, ethanol, and organic acids production. In the microbial challenge testing, all reference strains challenged into *tej* and *grawa* samples showed a gradual reduction. Among the strains, *E. coli* had the highest resistance next to *C. albicans* in two alcoholic beverages. Hence, the survival ability of the pathogens in alcoholic and low pH beverages (*grawa* and *tej*) was very low. Moreover, *borde* (the low alcoholic beverage) supports the growth of pathogens and cause health risks unless care is taken after preparation.

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## Authors' contributions

RN: Conceptualization, designing, validation, formal analysis, investigation, resources, data curation, writing—original draft. KB: Conceptualization, designing, validation, formal analysis, writing—review and editing, supervision, project administration, and fund acquisition. The authors read and approved the final manuscript.

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## Availability of data and materials

All data supporting the results are included in the article.

## Declarations

## Ethics approval and consent to participate

NA.

## Consent for publication

NA.

## Competing interests

The authors declare that they have no conflict of interest.

## Author details

<sup>1</sup>Department of Biology, College of Natural Sciences, Jimma University, P.O.Box 378, Jimma, Ethiopia. <sup>2</sup>Dambi Dollo College of Teachers Education, Dambi Dollo, Ethiopia.

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## References

- Adebijoyi JA, Kayitesi E, Adebo OA, Changwa R, Njobeh PB (2019) Food fermentation and mycotoxin detoxification: an African perspective. *Food Control* 106:106731. <https://doi.org/10.1016/j.foodcont.2019.106731>
- Almeida EG, Rachid CC, Schwan RF (2007) Microbial population present in fermented beverage 'cauim' produced by Brazilian Amerindians. *Int J Food Microbiol* 120(1-2):146–151. <https://doi.org/10.1016/j.jfoodmicro.2007.06.020>
- Antony U, Chandra TS (1997) Microbial population and biochemical changes in fermenting finger millet (*Eleusine coracana*). *World J Microbiol Biotechnol* 13(5):533–537. <https://doi.org/10.1023/A:1018561224777>
- AOAC (1990) Official methods of analysis, vol 1, 15th edn. Herich. Ked. Association of Official Analytical Chemists, Arlington
- Ashaolu TJ (2019) A review on selection of fermentative microorganisms for functional foods and beverages: the production and future perspectives. *Int J Food Sci Technol* 54(8):1–9. <https://doi.org/10.1111/ijfs.14181>
- Bahiru B, Mehari T, Ashenafi M (2006) Yeast and lactic acid flora of *tej*, an indigenous Ethiopian honey wine: variations within and between production units. *Food Microbiol* 23(3):277–282. <https://doi.org/10.1016/j.fm.2005.05.007>
- Chauret C (2011) Survival and control of *Escherichia coli* O157:H7 in foods, beverages, soil and water. *Virulence* 2(6):593–601. <https://doi.org/10.4161/viru.2.6.18423>
- Chaves-Lopez C, Rossi C, Maggio F, Paparella A (2020) Changes occurring in spontaneous maize fermentation: an overview. *Fermentation* Rev 6(1):36. <https://doi.org/10.3390/fermentation6010036>
- De Roos J, De Vuyst L (2018) Acetic acid bacteria in fermented foods and beverages. *Curr Opin Biotechnol* 49:115–119. <https://doi.org/10.1016/j.copbio.2017.08.007>
- Ekundayo FO, Oladipupo OA, Ekundayo EA (2013) Studies on the effects of microbial fermentation on bush mango (*Irvingia gabonensis*) seed cotyledons. *Afr J Microbiol Res* 7:4363–4367. <https://doi.org/10.5897/AJMR12.1800>
- Ewuoso MO, Animashaun OH, Adejumo AA (2020) Lactic acid bacteria and yeasts in spontaneously fermented sorghum sourdough. *Am J Microb Res* 8:63–72. <https://doi.org/10.12691/ajmr-8-2-4>
- Jeon S, Kim N, Shim M, Jeon Y, Ahn J, Lee SH, Hwang IG, Rhee M (2015) Microbiological diversity and prevalence of spoilage and pathogenic bacteria in commercial fermented alcoholic beverages (beer, fruit wine, refined rice wine, and yakju). *J Food Prot* 78(4):812–818. <https://doi.org/10.4315/0362-028X-JFP-14-431>
- Jespersen L (2003) Occurrence and taxonomic characteristics of strains of *Saccharomyces cerevisiae* predominant in African indigenous fermented foods and beverages. *Fed Eur Microbiol Soc* 3:191–200. [https://doi.org/10.1016/S1567-1356\(02\)00185-X](https://doi.org/10.1016/S1567-1356(02)00185-X)
- Kebede A (2007) Isolation, characterization and identification of lactic acid bacteria involved in traditional fermentation of *borde*, an Ethiopian cereal beverage. *Afr J Biotechnol* 6:1469–1478
- Ketema B, Tetemke M, Mogessie A (1998) The microbial dynamics of "Borde" fermentation, a traditional Ethiopian fermented beverage. *SINET:Ethiopl.Sci* 21(2):195–205. <https://doi.org/10.4314/sinet.v21i2.18120>
- Kim SA, Kim NH, Lee SH, Hwang IG, Rhee MS (2014) Survival of foodborne pathogenic bacteria (*Bacillus cereus*, *Escherichia coli* O157: H7, *Salmonella enterica* Serovar Typhimurium, *Staphylococcus aureus*, and *Listeria monocytogens*). *J Food Prot* 77(3):419–426. <https://doi.org/10.4315/0362-028X-JFP-13-234>
- Kirchmayr MR, Segura-garcía LE, Lappe-oliveras P (2017) Impact of environmental conditions and process modifications on microbial diversity, fermentation efficiency, and chemical profile during the fermentation of Mezcal in Oaxaca. *LWT Food Sci Technol* 79:160–169. <https://doi.org/10.1016/j.lwt.2016.12.052>
- Kosisochukwu C, Iyeoma F, Nkemjika G (2020) Microbial succession pattern in Ogi fermentation international journal of advanced research in biological sciences microbial succession pattern in Ogi fermentation. *Int J Adv Res Biol Sci* 5:1–6. <https://doi.org/10.22192/ijarbs>
- Laetitia M, Joseph HD, Joseph D (2005) Physical, chemical and microbiological changes during natural fermentation of "gowé", a sprouted or non sprouted sorghum beverage from West-Africa. *Afr J Biotechnol* 4:487–496
- Lemi BW (2020) Microbiology of Ethiopian traditionally fermented. *Int J Agric Food Sci*. <https://doi.org/10.1155/2020/1478536>
- Liang Z, Lin X, He Z, Li W, Ren X, Lin X (2020) Dynamic changes of total acid and bacterial communities during the traditional fermentation of Hong Qu glutinous rice wine. *Electron J Biotechnol* 43:23–31. <https://doi.org/10.1016/j.ejbt.2019.12.002>
- Lund P, Tramonti A, Biase D (2014) Coping with low pH: molecular strategies in neutralophilic bacteria. *Fed Eur Microbiol Soc* 38(6):1091–1125. <https://doi.org/10.1111/1574-6976.12076>
- Menz G, Vriesekoop F, Zarei M, Zhu B, Aldred P (2010) International journal of food microbiology the growth and survival of food-borne pathogens in sweet and fermenting brewers' wort. *Int J Food Microbiol* 140(1):19–25. <https://doi.org/10.1016/j.jfoodmicro.2010.02.018>

- Mora-Villalobos JA, Montero-Zamora J, Barboza N, Carolina R-G, Usaga J, Redondo-Solano M, Schroedter L, Olszewska-Widdrat A, López-Gómez JP (2020) Multi-product lactic acid bacteria fermentations. *Fermentation Rev* 6(1):23. <https://doi.org/10.3390/fermentation6010023>
- Mulaw G, Tesfaye A (2017) Technology and microbiology of traditionally fermented food and beverage products of Ethiopia: a review. *Afr J Microbiol Res Rev* 11:825–844. <https://doi.org/10.5897/AJMR2017.8524>
- Navarrete-bola L (2012) Improving traditional fermented beverages: how to evolve from spontaneous improving traditional fermented beverages: how to evolve from spontaneous to directed fermentation. *Eng Life Sci* 12(4):410–418. <https://doi.org/10.1002/elsc.201100128>
- Nemo R, Bacha K (2020) Microbial, physicochemical and proximate analysis of selected Ethiopian traditional fermented beverages. *LWT Food Sci Technol* 131:109713. <https://doi.org/10.1016/j.lwt.2020.109713>
- Nemo R, Bacha K, Ketema T (2017) Microbiological quality and safety of some-street- vended foods in Jimma town , southwestern Ethiopia. *Afr J Microbiol Res* 11(14):574–585. <https://doi.org/10.5897/AJMR2014.7326>
- Peyer LC, Zannini E, Arendt EK (2016) Lactic acid bacteria as sensory biomodulators for fermented cereal-based beverages trends in food science & technology lactic acid bacteria as sensory biomodulators for fermented cereal- based beverages. *Trends Food Sci Technol* 54:17–25. <https://doi.org/10.1016/j.tifs.2016.05.009>
- Shamebo T, Bacha K, Ketema T (2016) The growth potential and antimicrobial susceptibility patterns of salmonella species and Staphylococcus aureus isolated from mobile phones of food handlers and health care workers in Jimma town , Southwest Ethiopia. *Afr J Microbiol Res* 10(8):254–259. <https://doi.org/10.5897/AJMR2015.7621>
- Siedler S, Balti R, Neves AR (2019) Bioprotective mechanisms of lactic acid bacteria against fungal spoilage of food. *Curr Opin Biotechnol* 56:138–146. <https://doi.org/10.1016/j.copbio.2018.11.015>
- Simatende P, Henry T, Jabulani S (2015) Methods of preparation of Swazi traditional fermented foods. *J Ethnic Foods J* 2(3):119–125. <https://doi.org/10.1016/j.jef.2015.08.008>
- Tadesse G, Ashenafi M, Ephraim E (2005) Survival of *E. coli* O157: H7 *Staphylococcus aureus*, *Shigella flexneri* and salmonella spp . In fermenting 'Ó Borde ', a traditional Ethiopian beverage. *Food Control* 16(2):189–196. <https://doi.org/10.1016/j.foodcont.2004.01.011>
- Tamang JP, Watanabe K, Holzapfel WH (2016) Review: diversity of microorganisms in global fermented foods and beverages. *Front Microbiol* 7: 377. <https://doi.org/10.3389/fmicb.2016.00377>
- Tsafrakidou P, Michaelidou A-M, Biliaderis C (2020) Fermented cereal-based products: nutritional aspects, possible impact on gut microbiota and. *Foods Rev* 9(6):734. <https://doi.org/10.3390/foods9060734>
- Vivijis B, Aertsen A, Michiels CW (2016) Identification of genes required for growth of *Escherichia coli* MG1655 at moderately low pH. *Front Microbiol* 7:1–14. <https://doi.org/10.3389/fmicb.2016.01672>
- Vylkova S (2017) Environmental pH modulation by pathogenic fungi as a strategy to conquer the host. *PLoS Pathog* 13(2):1–6. <https://doi.org/10.1371/journal.ppat.1006149>

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