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Evaluating the probiotic potential of Yeasts isolated from Ethiopian traditionally fermented foods and dairy products

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Abstract

Fermented foods were most prepared products for their benefits of health such as medicinal, therapeutic and probioti c effects. These effects were due to potential probiotic microorganisms such as lactic acid bacteria and yeasts. The purpose of this study was to evaluate the probiotic characteristics of yeasts isolated from Ethiopian traditionally fermented foods and dairy products. The isolates identified and subjected to analyses to determine their probiotic properties. From the selected isolates ten were found to be resistant and showed good growth at pH 2, 2.5and 3for 24h and were able to grow at both 25, 37 and 45°C but best growth was found at body temperature 370C. The three isolates namely Y66, Y82 andY36 showed maximum growth at pH 2.5 with corresponding mean OD value of 1.45, 1.40 and 1.09. From the finding insolate Y77 and Y82 performed well at pH 2.5 and 3 compared to other isolates. The three isolates GB48, Y77 and Y82 shown highest growth rate with mean OD630nm value of 2.25, 1.85 and 1.78 respectively at 370C. Isolates were also investigated for bile salt tolerance, cholesterol assimilation, co aggregation and antioxidant abilities. IsolateY76 and Y82 were found to be with highest bile salt tolerant and cholesterol assimilation of 46.56, 56.20% and 96.11, 92.11% of assimilation respectively. The two isolates Y77 and Y82 also showed good survival rate of 44.59 and 36.56% in gastrointestinal fluid and antioxidant scavenging ability of 44.59 and 36.00% respectively. Among these isolates Y82, Y76 and Y77 have been found to bear with promising desirable probiotic potentials properties.

Keywords: Antioxidant; Co aggregation effect; Probiotic Yeast; Ethiopian

1. Introduction

Fermented food products are essential components of diet in a number of developing countries and are consumed either as beverage, main dish or condiment, which contribute to one third the diet of people worldwide [1]. Traditiona lly, fermented foods are processed through naturally occurring microorganisms; however, modern conventional meth ods of production generally exploit the use of defined starter culture to ensure consistency and the quality of the final product [2]. Cultures and species involved in fermented foods do not pose any health risk, and thus are designated as 'GRAS' (generally recognized as safe) organisms [3].Therefore, some of the species of these microorganisms because of their long history of safe use in food products can be implemented as protective cultures or probiotics.

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Probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [4]. Many different organisms are used as probiotics such as yeast, bacteria, and molds. The only probiotic yeast used are the non-pathogenic *Saccharomyces boulardii* and *S. cerevisiae* strains commonly used as probiotic yeast species and its application as bio-therapeutic or probiotic agents for re-equilibration of intestinal micro flora and current studies have demonstrated their efficacy in treating chronic or recurrent diarrhea associated with *clostridium difficilet* [5]. Yeast probiotics also include reduction in the incidences of diarrhea, constipation and bowel cancer, stimulation of the immune system, and enhanced nutrient uptake as it was suggested by [6]. Some studies by Adisa and Ifesan [7], indicated that yeast probiotics plays main role in reduction of hypercholesterolemia, an elevated level of cholesterol and becomes a major risk factor for cardiovascular diseases (heart attacks) thus it received much attention as a viable option to prevent cardiovascular diseases, considering the failure of the current strategies such as dietary management and the use of pharmacological agents.

Probiotic yeasts also demonstrate antagonistic activity against spoilage microorganisms, resist low pH and high salt concentrations, produce desirable aromas and improve lactic acid bacteria growth [8].These distinct attributes enable yeast to be considered as agents of probiotic because quite a number of species are able to survive passage through the gastrointestinal tract and show favorable effects on the host. With this features, yeasts may contribute to the improvement of the health of consumers by means of the production of vitamins and antioxidants, degradation of non-assimilated compounds (such as phytate complexes),inhibition of pathogens, decrease in cholesterol levels, adhesion to intestinal cell line and the maintenance of epithelial barrier integrity [9]. Some yeast strains such as *Saccharomyces cerevisiae* and *Saccharomyces boulardii* have been used as probiotics in humans for many years because they exert some influence on the intestinal flora [10]. Fermented foods are the common delivery system for probiotic microorgan isms. Given that fermented food products can contain probiotics, prebiotics or both, it is not surprising that their consumption has long been associated with good health. Therefore, yeasts obtained from traditional fermented foods and beverages of Ethiopia have not been explored with respect to their probiotic properties. Therefore, the present study was conducted to evaluate these yeast isolates obtained from traditional fermented foods of Ethiopia, and investigate for probiotic attributes (pH, temperature salt, cholesterol reduction, co aggregation and antioxidant) effect of the isolates.

2. Material and methods

2.1. Sample collection and isolation

About 20 samples were collected from various traditional fermented foods (*teff dough and kocho*) and fermented milk products (yogurt *and cheese*).Yeast was grown using yeast extract peptone dextrose (YEPD) media (1% yeast extract, 2% peptone, and 2% glucose/dextrose in distilled water) followed by serial dilution of 10^4 to10^6, then incubated at 28 °C for 24 48hrs according to the method described by [11]. Potential yeasts were isolated from the fermented food pro ducts and beverages using standard serial dilution methods, were selected and streaked again on YEPD agar plates for purification. All yeasts were preliminarily grouped based on their cultural morphology by microscopic and macroscop ic observations. The grown single colonies were stored at 4°C for further study. Purified yeast isolates were stored at −80°C as glycerol stocks for long term storage.

2.2. pH Tolerance

pH tolerance was tested using methods modified from [12]. Acid tolerance was evaluated by growing the yeasts in bro th adjusted to different Ph between 3.5 4.5 and 5.5 using 5M of HCl and 5M of NaOH, then incubated at 37°C for 24h.Ye ast growth was monitored using a spectrophotometer by reading the optical density at 630nm (OD_{630}) against un inoc ulated broth as the blank.

2.3. Temperature tolerance test

Cultures were incubated in YPD broth and incubated at four different temperatures at 25°C, 37°C and 45°C. Growth wa s observed spectrophotometrically at 0, 2, 4 and 24h of incubation, reading the optical density at 630 nm against un-in oculated broth with modified method of [13].

2.4. Bile salt tolerance

The ability to be probiotic yeast was known by regulating the bile salt tolerance test with the addition of synthetic bile salts on YPD broth medium as much as 2% 6% 8%. [14]. A control was set up by inoculating cells in MEA broth withou

t bile. The isolates were incubated at 37ºC for 24h. Absorbance readings at 630 nm were recorded and the growth sur vival calculated thus

$$\text{Percentage survival of isolate } = \frac{MEAc - MEAt}{MAEc}\ x\ 100$$

Where *MEAC* = control

MEAt = yeast isolates

2.5. Cholesterol removal test

About 2%, 6%ad 8% of bile salt and 100µg of water soluble cholesterol were added into YEPD broth adjusted to pH-2, then yeast isolates were inoculated and incubated at 37°C for different time intervals of 0, 4 and 24hr.5ml of culture w as taken in each in travel and centrifuged 5000 rpm for 5 minutes [15]. Cholesterol assimilation rate was measured usi ng supernatant optical density at 630nm. Growth of yeast isolates were measured at 630 nm to check the rate of chole sterol removal using the following formula to calculate the assimilation rate,

$$\frac{\text{concentration in control} - \text{cholos concentr in sample}}{cholestrol\ concentration\ in\ control!}x\ 100$$

2.6. Co-aggregation

Co-aggregation was estimated as described by [16]. Accordingly, culture was suspended in 0.2ml of PBS and mixed wit h equal amount of pathogen (0.5OD) and the final volume was adjusted to 5ml. It was then incubated for 24h at 37^0C. The OD at 630nm of the suspension were measured after 24h of incubation and compared with pathogen suspension. After 24h of incubation, yeast cultures were taken and stained by methylene blue and observed under microscope.

2.7. Survival in intestinal environment (intestinal juice)

Survival in intestinal environment was estimated according to [17]. Active yeast cells were harvested by centrifugatio n at 3000/g for 10 min, then inoculated at the level (10^6 cfu ml/1) into medium reproducing human intestinal conditio n, constituted by an aqueous solution containing 1g.l/1 in saline NaCl (0.5%, w/v), the pH being adjusted to 8.0 with 0. 1M NaOH before 2% glucose was added. The cells viability was determined by spectrophotometer reading at 630nm a fter 0, 1, 2, 3 and 4 hours of incubation at 37^0C.The results were got as the mean value of three replicates [18]

2.8. Antioxidant activity

Antioxidant activity of intracellular cell-free extract wet weight of yeast cell pellets was measured and suspended in 2 ml of phosphate buffer (02 moll 1, pH 7). Cell disruption was carried out with mortar and pestle on ice for 15 min. Cell debris was removed by centrifugation at 12000g for 10min at 4°C, and the resulting supernatant, which is the intracell ular cell-free extract, was used to assay for antioxidant activity [19]. The antioxidant activity was measured by using t he 1,1-diphenyl 2 picryl-hydrazyl (DPPH) free radical scavenging assay [20]. The percentage antioxidant activity was defined as follows:

$$\text{Antioxidant activity \%} = \frac{\text{A0}-\text{A}}{Ao}x100$$

Where A0: Optical density of in control,

A: Optical density of intracellular cell-free extract.

2.9. Statistical Analysis

Results were expressed as mean ± standard deviation. Statistical significance was calculated by ANOVA supported by Student's *t* test. Differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Isolation and identification of yeasts

Yeasts were isolated from different sources of fermented traditional foods. A total of 56 isolates that resembled yeasts with distinct colonies were appeared in the isolation plates. Out of the total isolated and purified cultures only twenty cultures, showed yeast cell morphology and selected for further studies.

3.2. pH and temperature and salt tolerance

Out of the twenty morphologically screened isolates only 10 yeast isolates GB19, GB48, Y36, Y60, Y63, Y65, Y66, Y76, Y77 and Y82 performed good growth at pH 2, 2.5 and 3 with corresponding growth value at (OD630nm) value in between 0.77 and 1.47 compared to the control 0.57 at (OD630nm) and other isolates. Out of those 10 isolates, the three isolates namely Y66, Y36 and Y82 performed good growth with their corresponding OD630nm value of 1.11, 1.09 and 1.07 respectively at pH 2. The three isolates Y66, Y80 and GB19 showed highest growth performance (1.20 &1.42, 1.17&1.37, and 1.09 &1.47 compared to the control (0.87 and 0.96) at pH 2.5 and 3 respectively. The growth performance of isolates decreased as the pH increased after 24h of incubation. This indicates that these isolates cannot grow well when the environment becomes more acidic. The above finding also indicated that the growth rate varies with time of incubation and pH (Fig1). Similar findings were reported by [21] that yeast can survive over wide pH range and have been reported from highly acidic environments like at pH 2.5. Similar results have been reached by using yeasts isolated from different fermented cereals which were capable of surviving and growing under stress conditions such as 2.0, 2.5 and 3.0 pH. Similarly, study conducted and reported [22] that yeasts isolated from chicken feces and kefirs showed high survival for 8h of incubation at pH 2.5. In this study, the selected yeast isolates namely Y 66 and Y82 survived at acidic pH 2 which was in line with the above findings. Since, one of the most important charact eristics for an isolate to be selected as probiotic yeast is its potential and performance to survive at low pH. Findings conducted by [23] also suggested another important criterion while selecting a potential probiotic is the tolerance of high acid levels in our stomachs and withstand at least pH 3.0

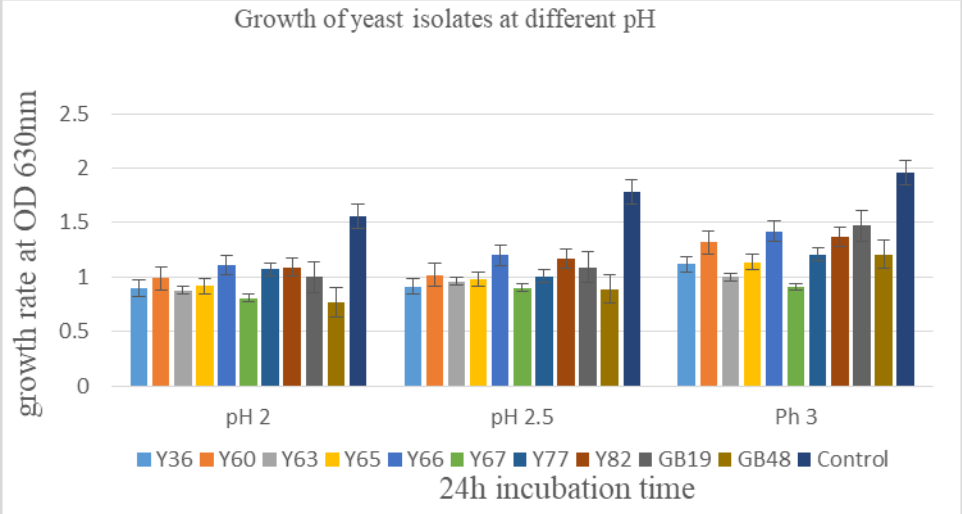

Figure 1 The growth performance of yeast isolates at different pH

The high temperature tolerance of the yeast isolates was presented in fig 2. At 250C, the growth rate at (OD630nm) of isolatesY66, Y36, and Y82 was 1.48, 1.46 and 0.88 respectively which was significantly different when compared to control (0.69) and the other seven isolates. At 37^{0}C the growth performance of three isolates namelyY76, Y77 and Y82 at 630nm was 2.12, 1.89 and1.86 respectively. The above value was higher when compared to other isolates and the control (0.72) which was grown in neutral conditions (pH 7). In general, the growth performance of yeast isolates achieved maximum at temperature 370C than the other two temperatures at25 and 45^{0}C and the three isolates Y76, Y77 and Y82 exhibited high temperature tolerance (Fig 2). Their growth rate was higher at 37°C but decreased when the temperature exceeds above 37°C. This implies that the growth characteristics of yeast isolates was best at human body temperature (37°C).This is in line with studies reported by [24] that the optimal temperature for most yeast growth is 28 30°C but a potential probiotic isolates must retain viability and metabolic functions active at body temperature (37°C).

The heat tolerance mechanisms indicated that the isolated yeasts were grown best at moderate temperature between 25°C and 45°C and showed good resistance towards moderate heat, which would be a positive property for these probiotics to be applied in food and feed industries. Similar results were reported previously for other yeast strains isolated from raw milk [25] cheese, and beverages [26]. Their growth rate is higher at 37°C but decreased when the temperature is above 40°C. Temperature tolerance of yeasts isolates Y76, Y77 and Y82 was one of the important characteristics of probiotic organisms and confirms an adverse condition. Therefore, the above tasted yeast isolates may survive in the human gastrointestinal tract and thus, create the possibility of proper activity in the human body.

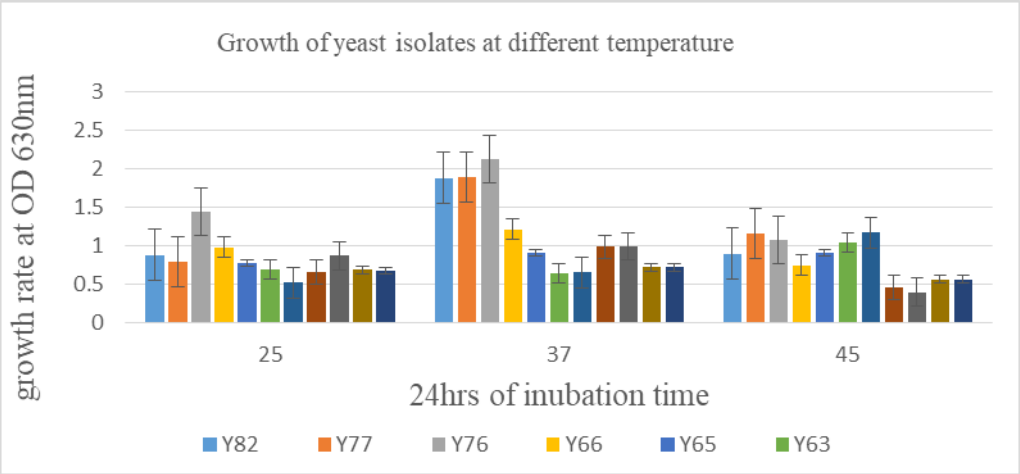

Figure 2 The growth of yeast isolates at different Temperature at 24h incubation time

Tolerance to bile salt is an important prerequisite for the colonization of the intestinal track, thus, the yeast isolates exhibited to bile salt with varying degrees of tolerance. The bile salts tolerance of the yeast isolates was shown in fig.3. All the isolates survived at different concentration of bile salts, but their survival ratio varies among the isolates. When compared to other isolates at 2% of bile salt concentrationY66, Y65 and Y76 were with the highest survival rate of 84.93, 84.73and 80.43% respectively, whereas, at 6% the highest survival ratio compared to other isolates was observed in three isolates Y76, Y65 and Y77 with their survival ratio of 79.52, 65.85 and 63.83% respectively whereas, the survival ratio of isolateY77 and Y82 at 8% of bile salt concentration was higher when compared to other isolates. From the finding in general the survival rate decreases with increasing percent of bile salt concentration.

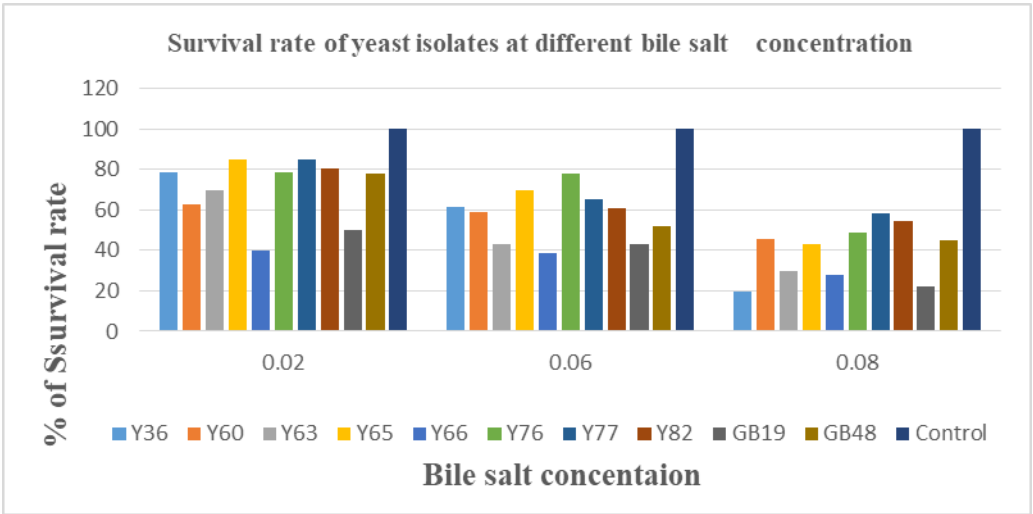

Figure 3 Salt tolerance of yeast isolates at different concentration level and time of incubation

Their capability of tolerating different concentration of bile salt by the yeast isolates may be attributed to their develo pment in harsh condition and makes them the potential candidates and offers their applicability as probiotics. The above mentioned isolates which withstand bile salt tolerance of 2, 6 and 8% were selected for probiotic properties since optimum bile concentration of our human gut environment ranges from 3% to 6%. This finding was in line with [27]

that certain yeasts with probiotic properties withstand bile concentration of 2% after 24h of incubation. Similar results suggested from the study conducted by [28] that yeast isolates from different fermented food samples with bile salt tolerance at 2% exhibited excellent probiotic potential. [29] also reported that the isolates succeeded to survive at 1% to 5% bile salts showed good properties of probiotics however on this result isolates Y66, Y82 Y76 and Y65 withstand the bile salt concentration of 2, 6 and 8% thus the selected isolates showed high survival ratio or resistant to bile salts. The survival capability of yeasts against bile salts was due to the presence of fatty acids and polysaccharides on cell wall which can reduce cell leakage against bile salts and improve the stability of membrane lipids [30].

3.3. Cholesterol assimilation and Co aggregation assay

High levels of serum cholesterol have been associated with the number of human diseases such as coronary heart disease thus using probiotic yeasts and bacteria plays a significant role in reducing serum cholesterol levels as it was suggested [31]. Cholesterol assimilation experiments revealed variation among the yeast isolates in their ability to remove cholesterol level. All isolates were able to lower cholesterol levels after 24h of incubation compared to the control. However, the amount of cholesterol reduction varied among isolates ranged from 11.24% to 75.11%. After 24hrs of incubation with cholesterol at 37ºC, the highest cholesterol assimilation was in isolate Y82 and Y76 with percent of reduction 74.11 and 71.11% respectively when compared to other isolates, whereas isolate GB19 assimilat ed the minimum amount (11.24%) of cholesterol (Table 1). Cholesterol reduction might be associated with the usage of cholesterol as source of nutrient for microbial growth. This is in line with results reported by [32] that the rapid cholesterol removal during 24h of incubation corresponded to exponential growth phase of the organisms. The possible reasons for reduction or assimilation of cholesterol were due to its assimilation during growth, incorporation of cholesterol into the membranes and binding to the cell's surface [31].Similar to these findings, cholesterol assimilation experiments carried out by [33] revealed a variation among the yeasts isolated from feta cheese and its percentage of removal ranged from 4% to 60% in different yeast strains. However, the removal range in this finding was greater (26.24 to 96.11%) than the above reported results (4-60%).

Table 1 Percent of cholesterol assimilation and co aggregative effect of yeast isolates with *E. coli*, and *L. monositogen.*

S/N	Isolate	Cholesterol assimilation (%)	Co aggregation effect with standard strain (%)	
			E. coli	***L.monositogen***
1	Y65	49.51	76.33	78.66
2	GB48	59.44	82.51	39.56
3	Y77	75.11	61.00	77.66
4	Y63	65.11	79.35	79.33
5	Y82	74.11	87.50	98.00
6	GB19	11.24	62.00	33.52
7	Y76	71.11	51.00	77.50
8	Y36	59.77	76.33	51.00
9	Y60	68.44	78.33	98.72
10	Y66	65.77	98.35	81.05
11	Control	76.47		

Yeasts are well studied probiotics to play a crucial role in the treatment of different inflammatory bowel disease reduce the duration of disease. The co aggregation assay is a reliable method to evaluate the close interaction between probiotic microbes and pathogenic bacteria. The co-aggregation ability of the yeast isolates in these findings associate d with *E. coli* and *L.monositogen* was shown in Table 1. Isolate Y66 and Y60 showed the highest co-aggregation effect of 98.35 and 98.72% with *E. coli* and *L.monositogen* respectively. The minimum co aggregation effect was observed on isolate GB19 and GB48 with co-aggregation effect of 51.00 and 39.56% with *E. coli* and *L.monositogen* respectively. In general, from the finding the co aggregation effect of yeast isolates with the two pathogenic organisms of *E. coli* and *L. monositogen* ranges between 51.00 - 98.35% and 33.52 98.72% respectively. The co-aggregative effect of all isolates with *E.coli* and *L.monositogene* was more than 50% however the co-aggregation effect of the two isolates GB19 and GB48 with *L.monositogene* was below 50%. The capability of forming aggregates is one of the most desirable character

istics of a potential probiotic microorganism, because aggregation of microorganisms affects microbial adherence to the intestines, thus providing potential competitive advantage in the colonization of the GI tract [34].

3.4 Survival rate of yeast isolates in intestinal environment and their antioxidant activity.

The intracellular cell free extract of the probiotic yeast isolates showed different ability to scavenge DPPH free radicals in a methanol reaction system (Table 2) shows an antioxidant activity in the range of 1.31% to 44.59% by the yeast isolates during 1 4hrs of incubation. Isolate Y77was with the highest scavenging percentage of 24.29, 32.77, 36.28 and 44.59% of DPPH free radical in methanol reaction systems during 1, 2, 3 and 4hs of incubation time respectively. From the finding in general as the time of incubation exceeds the scavenging capacity of the yeast isolates increased. The scavenging capacity of the above yeast isolates were higher than the previous results reported by [31] who analyzed yeast cells from different food products, with a percentage of scavenging activity ranging from 4.25 to 46.78%. The antioxidant activity of yeast isolates was mainly due to the high content of β glucans found in the cell wall, and cellular compounds of antioxidant enzymes which include superoxide dismutase, glutathione peroxidase and catalase as it was reported [32].

The free radical scavenging potential of the current yeast isolates might be due to the presence of intracellular antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase. The antioxidant ability also associated with the production of certain compounds on the cell surface, including extracellular polysaccharides [35]. The results of the present study indicated that the radical scavenging ability of yeast isolates varies with time of incubation towards the ant oxidative effect

Table 2 % of DPPH scavenging capability and Gastric juice tolerance of the yeast isolates.

S/N	Isolate	Percent of scavenging DPPH at different incubation time				Gastric juice tolerance at different incubation time			
		1	2	3	4	1	2	3	4
1	Y65	11.76	6.07	8.34	17.07	12.33	6.07	7.21	5.69
2	GB48	4.19	4.57	6.28	8.57	3.61	4.57	6.28	8.57
3	Y77	24.29	32.77	36.28	44.59	23.12	32.77	30.28	44.59
4	Y63	21.44	23.52	29.44	24.32	21.44	23.52	29.44	24.32
5	Y82	11.82	20.17	22.43	36.00	11.82	20.17	21.91	33.56
6	GB19	7.52	15.04	20.91	29.72	7.52	15.04	21.10	28.62
7	Y76	1.31	3.00	5.45	6.39	3.75	4.22	5.45	6.39
	Y36	2.31	3.49	4.01	5.77	5.11	6.49	6.87	7.11
8	Y60	17.96	18.64	21.01	21.01	17.96	18.64	20.34	21.35
9	Y66	2.18	3.37	8.13	7.34	7.33	8.45	12.96	10.52

The effects of *in vitro* simulated gastric condition on tolerance of yeast isolates were showed in (Table. 2). Since it is an important property of probiotic microorganisms which enter the GI tract must resist gastro intestinal enzymes in addition to low pH and bile salt. During this investigation, survival of intestinal environment assay was carried out for the determination of the capacity of the tested yeasts to overcome the stress conditions of the intestinal habitat through their growth rate. It can be observed from Table 2 that the survival percentage for yeast isolates ranged from 3.75% (Y76) to 44.59% (Y77) of tolerance during 1 4h of incubation.

According to the present obtained results maximum tolerance rate was revealed by isolate Y77and Y82 with its corresponding tolerance percentage of 44.59 and 33.56% respectively after 4h of incubation whereas, isolateY65 shown minimum tolerance rate of 5.69 % (Table 2). In general, the rate of growth increased with the time of incubation but isolate Y63, Y65 and Y66 tolerance rate decreased from 29.44, 7.21 and 12.96% after 3h of incubation to 24.32, 5.69 and 10.52% after 4h of incubation respectively. These finding indicated that Y77and Y82 could withstand the acidic stomach environment better than the other isolates and expected to provides its beneficial activity when

applied thus it could be suggested as potentially probiotic yeast. This results was in agreement with studies suggested by [36] that food borne yeast isolates demonstrated high tolerance to simulated human upper gastrointestinal tract juices, and thus they offer a relatively overlooked source of potential probiotics, apart from bacteria.

4. Conclusion

Fermented traditional foods are considered as the main source of beneficial probiotics. Although, there were number of studies carried out on lactic acid bacteria the use of yeast as a probiotic food supplement nowadays get attention. Ethiopian traditional fermented foods and beverages were an excellent source of probiotic yeast isolate. In this study, from 52 yeasts isolated from traditional fermented food products, isolate Y82, Y76 and Y77 have showed potential probiotic properties with their capability to survive in the acidic pH of stomach and human intestine and provides a potential probiotic properties at human body temperature. The three isolates also revealed good cholesterol assimilati on, suppress some foodborne pathogens as well as good antioxidant potential. Thus, they could be used as a potential yeast probiotic in food supplements.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have no conflicts of interest to disclose.

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