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Evaluation of Lactic Acid Bacteria Isolated from Ethiopian traditional fermented food ("Borde") and beverage ("Ititu") as a Starter Culture for Ayib Production.

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Abstract

In Ethiopia there are a lots of traditionally fermented foods and beverages widely produced and consumed among the communities and they have used for different purposes like ceremony, wedding and inviting of respected gusts. Mostly these traditional activities has been controlled with the activities of normal folar of the ingredients used for the preparation without addition of functional starter culture. Ayib is one of the widely used Ethiopian traditional fermented milk products, but mostly among the rural communities the ayip production is not under controlled environment, in developed countries production of fermented foods is based on the use of starter cultures, for instance lactic acid bacteria that initiate rapid acidification of the raw material. In Ethiopia the use of starter culture is not a common practice. Hence, the present study was conducted at Wollega University to evaluate the effect of lactic acid bacteria isolated from Ethiopian traditional fermented food and beverages (Ititu and Borde) to evaluate their potential activity as starter culture for Ayib Production. The samples were collected and transported to Wollega University Microbiology Laboratory for isolation and characterization of the potential starter culture and totally about 94 LAB were isolated, out of these 66 of them were selected as a presumptive LAB and further categorized based on morphological, biochemical and physiological activity. These isolates were used for production of ayib as of starter culture for ayib. Sensory analysis of ayib were conducted and result showed that cheeses made from all starter cultures received, in general higher scores for body texture, flavor and overall acceptability than cheeses made from control ($p<0.05$). From over all analysis the best acceptable and quality cheese was produced from mixed cultures with equal ratio of both isolates. Hence, the LAB isolates (Lactobacillus and Lactococcus) species were selected as the best starter culture for the production of ayib. The study generally includes evaluation of soft cheese made from different cultures and milk samples. As a result further study is recommended to determine the optimum mix of the starter cultures for best quality and shelf life under economically feasible production system.

Keywords: soft cheese, traditionally fermented foods, lactic acid bacteria, starter culture

1. Introduction

Traditional fermentation which involved microbial activity has been used for long period of time and the technology has been utilized in food production, food processing and preservation as the sole advanced technology. Lactic acid bacteria (LAB) are widely utilized to produce fermented foods, contributing to flavor development as well as safe metabolic activities because, they are recognized as Safe (GRAS). In addition, they produced various antimicrobial metabolites (lactic acid, acetic acid and other organic acids, hydrogen peroxides, bacteriocins and bacteriocin like substance) that have important role in the inhibition of food borne pathogenic and spoilage microorganism to enhance for food safety and quality besides nutritional enhancement (*Cadirici and Citak*, 2005). Fermentation with lactic acid bacteria (LAB) is the effective food preservation method that can be applied even in more rural or remote places and lead to improvement in texture, flavor and nutritional value of many food products. LAB has a long and safe history of application in cheese processing (*Aquilanti et al*., 2006). Some strains of LAB may contribute to food preservation of fermented food by producing bacteriocins (*Brink., et al,* 1994*).* The major parameter involved in bacterial growth inhibition is the pH, which decreases by the production of organic acid, nutrient competition, hydrogen peroxide and antibiotic production.

In dairy industry a number of LAB have been used as starter cultures, due to their essential roles in production of lactic acid which imparts a distinctive fresh and acidic flavor during manufacture of fermented milk. This lactic acid is also important in cheese making during the process of coagulation and texturization of crud and the starter cultures may possess proteolytic and lipolytic activities which may be desirable especially during the maturation of certain types of cheese. Finally, acidic condition in these products prevent the growth of pathogens as well as many spoilage organisms (*Tamime,* 1981*).*

In Ethiopia, Ayib is produced from sour skim milk. The use of LAB isolated form fermented milk and cereal products could enhance the quality of the product, shelf stability and safety. However, careful selection and evaluation of such LAB isolates for use as a starter culture is the first step in the right direction. Hence, this study was conducted isolate and chatractetizatize, evaluate the potential activity of lactic acid bacteria as ayib starter culture (Ethiopian Cottage cheese) and to assess the microbiological, chemical quality as well as sensory property of the produced Ayib.

2. Materials and Methods

The study was conducted in Wollega University, Nekemte town, the traditional fermented food (*Ittitu)* and beverage (*Borde*) were collected from two district of East Wollega Zone of Oromia Regional State, Western Ethiopia (Nekemte town and Sibu Sire Woreda). For each sample about 500ml sample were collected from ten selected households from each district, during November 2015- January 2016. The samples were collected using sterilized flask and kept under refrigerator temperature using an ice box and brought to the microbiology laboratory of biology department of Wollega University. The samples were kept in refrigerator until analysis.

2.1 Sample preparation

Ethiopian traditional fermented food (Ititu) and beverage (borde) were collected from Nekemte town and Sibu sire woreda using sterilized flasks and brought to the Microbiology laboratory, Department of Biology, Wollega University. One ml of each sample were taken and added separately in to test tube containing 9ml of normal saline (0.85% W/V) solution and mixed well using vortex mixer. Appropriate serial dilution were made up to 10^{-7} and 0.1 ml suspension were taken and spread on MRS agar and the plates were incubated an anaerobically at room temperature for 24hr-48hrs days in an anaerobic jar and aerobically.

2.2 Isolation of lactic acid bacteria

Enumeration of lactic acid bacteria was carried out after plating well diluted (10^{-7}) 0.1ml of the sample on the Mann Rogosa Sharp agar (MRS) and incubating an anaerobically in anaerobic jar (BBL, Gas pak plus) at $37C^{0}$ for 48hrs. Five to ten colonies were randomly selected from countable MRS Agar plate replica .The colonies were purified by successive streaking on appropriate agar media (MRS) before being subjected to characterization. Five colonies with different morphology from each plate were transferred to MRS broth, incubate for about 24hours and maintained in the refrigerator at 4°C. The isolates were grouped to their respective genus after examining for their gram reaction, cell and colony morphology, catalase reaction and gas production from glucose

fermentation. Those that are characterized as lactic acid bacteria were kept as a stock culture in the refrigerator at -20°C in a glycerol solution.

2.3 Characterization of lactic acid bacteria

2.3.1 Morphological characterization

To determine the morphological characterization of LAB, gram reaction, cell shape and motility were conducted following the standard microbiological producer.

2.3.2 Biochemical and Physiological characterization of LAB

Biochemical characterization like catalase test, oxidase test, spore testing and the survive potential of LAB isolates to stress conditions like temperature, salt concentration and different acid concentration was evaluated. Furthermore, the ability of LAB to ferment different carbon sources as source of energy was evaluated.

2.4 Isolation of Pure Culture of LAB

The LAB isolated from *Ititu* and *Borde* were further characterized based by their cell morphology, physiology and biochemical test grouped as gram positive, catalase negative, ccocci, cocco-bacilli or rod shaped, with characteristics cell arrangement (*Savadogo.et al* 2008).

2.5 Separation of Cream from the Fresh Whole Milk

Two liters of fresh cow milk were collected using autoclaveable plastic container which has been pre-sterilized at 120^0c for 15 min, and kept ready for milk collection from Wollega University farms. Centrifugal cream separator was used to separate the cream. The skim milk was heat treated at 100%C for 5 min and kept for use.

2.6 Selection of LAB as starter culture

A well- isolated colony was selected from MRS agar plate culture. The milk culture was then incubated at 35^0c for about 24hrs. A 24 hrs pure culture of LAB were introduced in to the skim milk which is heat treated at 100^0c for about 5min. Three drops of culture milk which contain the isolated LAB starter culture was added in to 150mlof skim milk to evaluate the strains of bacteria for fermentation rate and quality property compared with the control groups, which is fermented without the addition of bacterial starter culture.

2.7 The Ayib Production

The skim milk was inoculated with the selected LAB and incubated at 25c for 24 hrs and the whey was drained. The produced Ayib samples were kept in the microbiology laboratory of the Biology department of University of Wollega and kept at $\leq 5^0$c until analysis was carried out. During the analysis the glass were aseptically opened and sensory, chemical and Micro biological analysis were carried out. Samples for microbial and chemical analysis were taken aseptically prior to sensory evaluation.

2.8 Sensory Evaluation of the Ayib

The sensorial qualities of the produced *Ayib* by using starter culture and the control, without starter culture were evaluated by characteristics profile. The sensory panels were composed of five non-trained tasters familiarized with Ayib.

2.9 Microbial Analysis of the Ayib

The microbiological qualities of the Ayib sample were evaluated over a period of two week by keeping at room temperature and under refrigerated storage at 4^oC and total coliform, yeast and mould count was made to evaluate the microbiological quality of ayib produced with different starter cultures.

2.10 Chemical Analysis of the Ayib

The chemical composition of the Ayib sample produced was evaluated over the storage periods like for fat content, ash content, protein content and moisture contents were determined by Gerber methods (*AOAC*, 2000).

2.11 Statistical Analysis (Data Analysis)

The collected data from the survey was analyzed by using descriptive statistics and analysis of variance, and mean comparison procedures of the statistical package for social science (spss 21 version).

3. Results and Discussion

3.1. Isolation and characterization of LAB

Results showed that one hundred thirty seven (137) LAB isolates were obtained, 66 of them were grouped as Lactic acid bacteria (30 of them were *Lactobacillus*, 25 of them were *Lactococcus*, 4 of them were *Pediococcus* and 6 of them were *Leuconostoc* spp., respectively (table 1).

Table 1. Lactic acid bacteria isolates collected from two districts (Sibu Sire and Nekemte) of East Wollega Zone.

Sample	LAB code	Expected LAB genera
Ititu	E2,E4,E6,E9,E10,E11,E12,E13,E23,E24,E40,E41,E42,E46 E39,E40, E11,E13	*Lactobacillus spp.*
	E15,E16,E20,E21,E22,E23,E25,E26,E27,E28,E29,E30,E41,E44,E45,	*Lactococcus spp.*
	E31,	*Leuconostoc spp.*
	E06,E07,	*Pediococcus spp.*
Borde	B2,B4,B9,B10,B12,B14,B18,B19,B37,B38, B26,B27	*Lactobacillus spp,*
	B15,B18,,B19,B20,B21,B25 B28,B29,B32,B34,	*Lactococcus spp.*
	B25,B26,B35,B36,B38	*Leuconostoc spp.*
	B20,B21	*Pediococcus spp.*

3.2. Morphological, biochemical and biochemical characterization of LAB

Totally, one hundred thirty seven (137) LAB isolates were primarily identified from each fermented food and beverage samples and cultured on MRS agar anaerobically for 48 hours at 37°C.Finally, sixty six (66) isolates were identified as presumptive LAB strains based on gram status, catalase reaction and other biochemical tests. The LAB isolates were classified into the genera *Lactobacillus Lactococcus, Pediococcus, Leuconostoc and Streptococcus* based on their morphology and biochemical characters. The predominant species was *Lactobacillus species* .The differentiating characteristics of all LAB species *are* given in table 1.

According to the result, *Lactobacillus* was the first the most dominant lactic acid bacteria in this study followed by *Lactococcus*, *Leuconostoc* and *Pediococcus* spp. (table 1). In line to the present study, Abdulkadir *et al*. (2011) reported that *lactobacilli* were dominant, from Ergo (traditional fermented milk) samples. Eyassu *et al*. (2012) also reported that *Lactobacillus* species isolated from Itiitu (fermented camel milk) was the dominant genus which comprised about 58% of the total LAB isolates followed by *Lactococcus* species which accounted for 25% and actually the study is much similar with the present study since the repeat the same samples with time and study are variation. In additionally, Girum *et al.* (2005) reported that LAB isolated from ready to consume Borde were tentatively grouped into *Lactobacillus* (60 isolates), *Leuconostoc* (15 isolates), *Pediococcus* (18 isolates).

Next , the most dominant genera isolated from the current study was, *Lactococcus*, it was the second most dominant lactic acid bacteria, while *Pediococcus* was the least LAB isolated from this study. As previous study conducted by Senait Zewdie *et al* (1995) in Ethiopian confirmed that, *Lactococcus is* oneof the most generalcommonly dominated in siljo fermentation and in KunuZaki, Nigerian Sorghum based fermented beverages. Thethird most dominant LAB group from this study was *Leuconostoc*. This result is similar to finding reported by Tetemke and Mogessie (1995); Ogbonnaya and Chidinma (2012); Oyedeji *et al*. (2013) and who showed that *Lactobacillus* as predominant LAB from siljo, Nigerian traditional fermented food, and *akamu* respectively. Besides to these above mentioned, plant based fermentation and traditional fermented goat milk was also predominantly carried out by genus *Lactobacillus* (Mugula *et al.*, 2003; Yelnetty *et al.*, 2014).

3.2. Physiological characterization of LAB isolates

Regarding to gas production from glucose, almost majority of the isolates, 40(60.60%) of them were relatively hetero-fermentative because they produced both gas and acid from glucose and while the rest of them were homo-fermentative which produce only acids from glucose.In relation to the fermentation ability of LAB isolates, out of the 66 isolates, 40 isolates (60.60 %) were homo-fermentative and other isolates, 26(39.39%) were considered as hetero-fermentative based on their glucose fermentation profile (Table 2). This result is toughening with several study conducted in Ethiopia. For instance, Ketema *et al.* (1999) found that the most dominant lactic flora comprised of both hetero-fermentative and homo-fermentative *Lactobacillus* spp. and that homo fermentative LAB predominated after 24 h of Shamita fermentation. Asnake and Mogessie (2010) reported that about 94% of the LAB isolated from Awaze, Qotchqotcha and tef dough was homo-fermentative while 6% of the isolates were hetero-fermentative.

Table 2. Morphological, biochemical and physiological characterization of LAB isolated from “Ititu” and “Borde” for the ayib production as potential starter culture.

Characteristics	*Leuconostoc*	*Lactobacillus*	*Lactococcus*	*Pediococcus*
Cell morphology				
Shape	Coccus	Rods	Coccus	Coccus
Arrangement	Tetrads in pairs Short chain	Pairs or short chain	Spherical /round	Tetrads in pairs/ short chains
Biochemical tests				
Gram reaction	+	+	+	+
Catalases	-	-	-	-
Oxidase	-	-	-	-
Motility	-	-	-	-
Endospore	-	-	-	-
O/F test	Fermentative	Fermentative	Fermentative	Fermentative
No. of isolates	6(9.09)	30(45.45%)	26(39.39%)	4(6.06%)
Fermentation				
Gas production	+ve,	+ve/-ve,	-ve,	-ve,
Glucose	+ve	+ve	+ve	+ve
Galactose	+ve	+ve	+ve	+ve
Lactose	+ve	+ve	+ve	+ve
Sucrose	+ve	+ve	+ve	+ve
Starch	+ve	+ve	+ve	+ve
Homo-fermentative, 26(39.39%)	-	16	6	4
Hetero-fermentative, 40(60.60%)	30	10	-	-

Table 3. Evaluation of growth of LAB isolates as a starter culture in skim milk (Fermentation time of skim milk in hours)

	Fermentation time in hours					
LAB isolated	6hr	12hr	18hr	24hr	36hr	48hr
Lactobacillus spp.	-	-	+	+	+	+
Lactococcus spp.	-	-	+	+	+	+
Pediococcus spp.	-	+	+	+	+	+
Leuconostoc spp.		+	+	+	+	+
Lactobacillus spp.+ Lactococcus spp.		+	+	+	+	+
Lactobacillus spp.+ Lactococcus spp. Pediococcus spp.	-	-	+	+	+	+
C1(control)	-	-	-	-	+	+

3.3. Rate of Fermentation

To determine the potential of Lactic Acid Bacteria as starter culture for the Production of Ayib, the fermentation rate was conducted at different temperatures and time and isolated Lactic Acid bacteria were evaluated for their potential fermentation. Therefore, *Lactobacillus* &*Lactobacillus* and their combination were found to be the most potential and promise isolate to produce Ayib. While *Lactobacillus*&*Lactobacillus* with *Leuconostc* and *Pediococcus* combination did not shown effective fermentation as of the two combination (Table 4). Then those combinations (*Lactobacillus* &*Lactobacillus*) having favorable processing and organoleptic quality can be used for large-scale production.As the result reported by M. *Ashenafi* (2006), the controlled fermentation on pasteurized milk is necessary especially with combinations having favorable processing and organoleptic quality can be used for large-scale production in consideration with fermentation time. Thus, effect of incubation temperature on acidity change during fermentation of milk was observed. Reducing temperature slowed down the acidification process (Gonfa, *et.al.* (2001) if the temperature is too high, fermentation will be rapid and over-souring occurs, causing a separation of the liquid and solid phase and gas production, thus leading to deterioration of appearance and texture (Gonfa, *et al.*, 2001). As the temperature of incubation was raised, the rate of pH drop was faster and the time of coagulation became shorter.In general, 40–45°C was the optimum growth condition for the mixed culture and the short incubation method. The actual fermentation stage can take place either in the retail container for the production of the milk is incubated in bulk for the manufacture of stirred yoghurt. Several authors have stated that medium composition either carbon source, nitrogen source, or ion source are important parameters in EPS biosynthesis (Zouari, 1992). Mostly, as the current study revealed that, most of the isolated Lactic acid bacteria ferment glucose starting at hr18,24hr,36hr and 48hr mostly at the last two hours 36hr and 48hr because the complete oxidation of glucose with acid, gas and acid was success at these time. Regarding to the rate of fermentation, 36hr and 48hr are the most promising and the most temperature at which the microbes highly ferment and produce high product, therefore these time are selected as the most promising fermentation rate.

Table 4. Mean Microbiological Count of the Ayib (cheese) samples (CFU/ml log 10CFU)

Samples	Isolates	RD	TAMC	TCC	TYC	TMC
Ayib produced by isolated starter culture	Lactobacillus spp.	1	ND	ND	ND	ND
		7	ND	ND	ND	ND
		15	ND	ND	5.5±0.49	1.33±1.33
	Lactococcus spp.	1	ND	ND	ND	ND
		7	ND	ND	ND	ND
		15	1.3±1.33	ND	5.5±0.36	ND
	Lactobacillus spp. + Lactococcus spp.	1	ND	ND	ND	ND
		7	ND	ND	ND	ND
		15	0.66±0.57	ND	3.25±1.64	1.33±1.33
Ayib produced without starter culture	Control	1	ND	ND	ND	ND
		7	5.3± 0.40	ND	6.24±0.82	3.15±1.58
		15	5.9±0.40	ND	6.24±0.64	5.06±0.290

ND=Not detected

TAMC=Total Aerobic Mesophlic Count, TCC= Total Coliform Count, TYC=Total Yeast Count and TMC=Total Mould Count.

Lactobacillus spp (Ayib sample made by addition of starter culture (only Lactobacillus). **Lactococcus spp** (Ayib sample made by addition of starter culture (only lactococcus spp)). **Lactobacillus spp. + Lactococcus spp** (Ayib sample made by addition of mixture of starter culture (with the combination of Lactobacillus and lactococcus spp). **Control (**Ayib sample made without the addition of starter culture (control group).

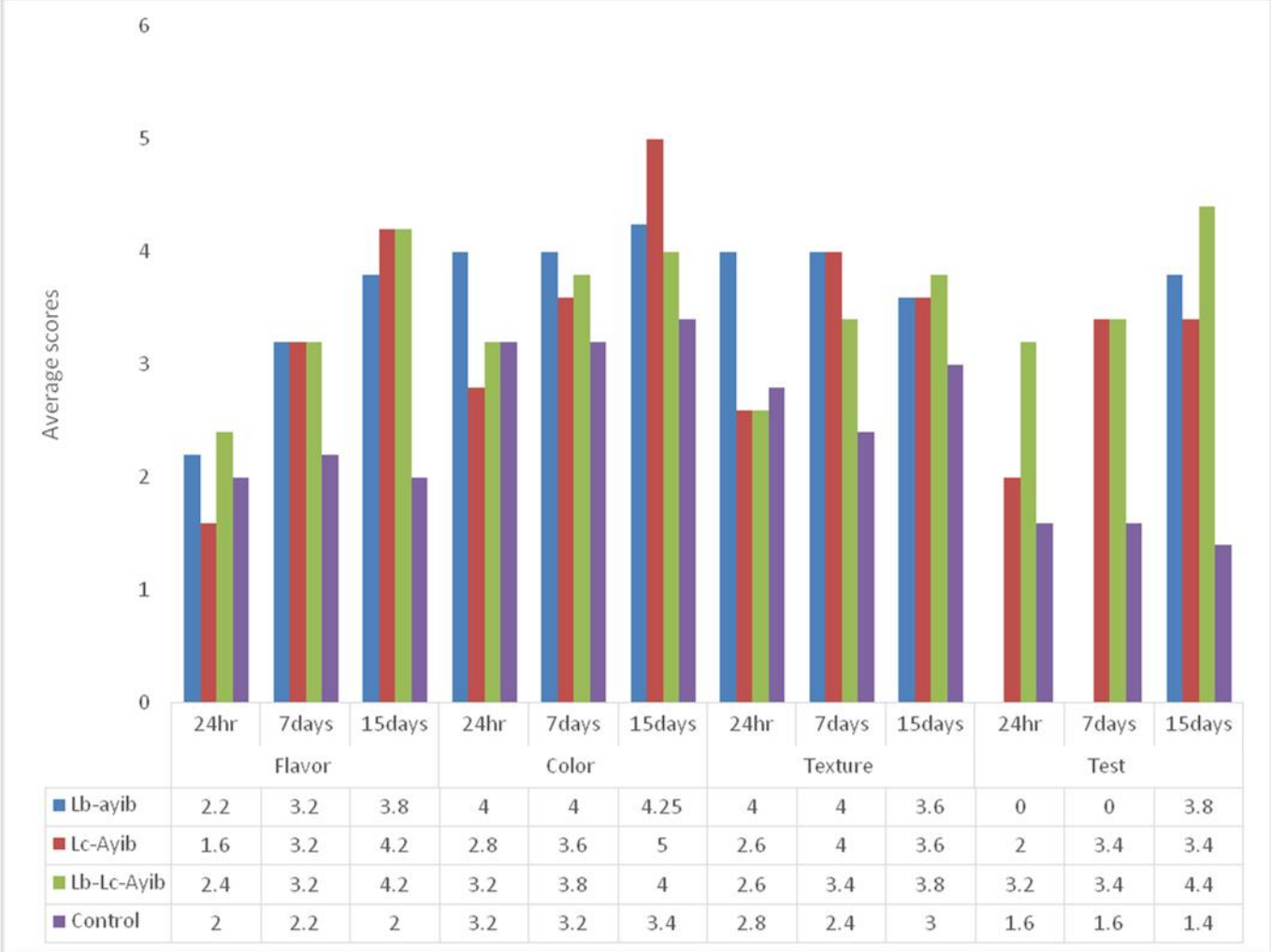
3.4. Microbiological Analysis

Regarding to the microbiological analysis of Cheese produced both from Starter culture and commercial (control culture), there was no any Total Coliform and Aerobic Mesolphilc counts. As the present study, the microbial counts of all samples were almost below detectable level. The finding is lower than the finding of Zelalem *et al.* (2007), who found 4.4, 5.1 and 7.9 log CFU /g of coliforms, and total bacterial count from *Ayib*, respectively; and also Ashenafi (1990) found 8log CFU /g of total bacterial count in Ayibsamples collected from an open market. This lower number of Aerobic mesophilic count and total coliform count might be due to microbiological quality and composition of milk types and water used for cleaning processing utensils (Marth and Steele, 2001). In addition, cleanness of the milking and processing utensils (Almaz *et al*., 2001). In addition, absence of microbial load from cheese is due to the activity of lactic acid bacteria as a result of production and presence of some potential antimicrobial compounds produced while fermentation process. This shows that, the Ayib (cheese) produced with the addition of starter culture is highly resistance to microbial contamination by bacterial rather than yeasts and molds.

Comparing the results obtained, the mold and yeast counts from each cheese samples were found to be higher. This could be as a result of unhygienic condition during milk reception, transportation and processing. From microbiological analysis it can be concluded that all cheese samples made from all proportions did not exceed the limit and it is inacceptable range. So, generally the cheese product in the present study has good shelf life and quality regarding to microbiological quality analysis of cheese.

3.5. Effect of LAB on shelf stability of cheese

To determine the effect of LAB on shelf satiability of the Ayib, sensory evaluation was employed and presented in figure 1. The sensory evaluation was conducted at 24hrs, 7 days and 15 days.



	24hr	7days	15days	24hr	7days	15days	24hr	7days	15days	24hr	7days	15days
	Flavor			Color			Texture			Test		
Lb-ayib	2.2	3.2	3.8	4	4	4.25	4	4	3.6	0	0	3.8
Lc-Ayib	1.6	3.2	4.2	2.8	3.6	5	2.6	4	3.6	2	3.4	3.4
Lb-Lc-Ayib	2.4	3.2	4.2	3.2	3.8	4	2.6	3.4	3.8	3.2	3.4	4.4
Control	2	2.2	2	3.2	3.2	3.4	2.8	2.4	3	1.6	1.6	1.4

Figure1. Shelf life enhancement of ayib using different species of Lactic acid bacteria isolated from ititu and borde, Ethiopian traditional fermented food and beverage.

The flavor score increased over time during the 15 days of storage for the Ayib produced with isolated starter cultures, while it remained the same for the control. .. It can be seen that, in the sensory profiles of cheeses, the improvement in colour from month zero to months 9 agreed with the findings of Tarakci and Kucukoner (2006), who reported that colour score increased generally during ripening. Flavor of all samples improved with storage months because during ripening the metabolic processes are responsible for the basic flavor and texture changes (Smit *et al*. 2005).

Similarly, the sensory evaluation of Ayib in relation to color score was investigated and determined over duration of two weeks and the result showed that Ayib produced with the use of *Lactobacillus* and *Lactococcus* species with their mixtures starter culture exhibited a higher color score than the stored for 15 days.

The chewiness of the cheese samples increased with the progressive ripening days and it was a highly desirable sensory attribute especially for cheese. The increase in chewiness might be due to a change in the average size of fat globules, distance between fat globules and variation in the size of globules (Richardson & Booth 1993). The improvement in flavor was probably attributed to the effect of lactic acid development which controls the growth of undesirable organisms (Kosikowski 1997). The improvement in flavor might be due to the natural flora initially present in milk which participates in flavor production. It is to conclude that the storage period significantly affected the weight loss, chemical composition and sensory characteristics of cheese.

3.6. Sensory quality

Ayib produced using isolated starter cultures had higher sensory scores by the taste panel when compared with the control group. Most of the differences found could be due to the addition of starter culture and lactic acid bacteria which uses the available proteins as a nutrient for the production of metabolites. The addition of starter culture allows the development of a well-fermented product with a significant increase in the quality of the taste and in the quality of the texture. As a result the presence of fiber particles alters cheese structure but when the fiber dose is high water absorption compensates the weakening effect of the fiber. This situation may be attributed to increased water holding capacity of milk proteins.

Table 5. The effect of LAB isolated on the chemical composition of Ayib

Sample	Fat %	Protein %	Ash %	Moisture %
Ayib prepared with Lactobacillus spp.,only	1.84±0.02	9.8±0.34	0.75±.01	81.1± 0.53
Ayib prepared with Lactococcus spp.,only	2.78±0.33	10.2±.0.36	0.78±.03	80.9±0.45
Ayib prepared with both Lactobacillus spp+ Lactococcus spp.	2.9±0.49	10.1+0.33	0.85±0.66	79.87±3.6
CONTROL	1.78±0.09	4.2±1.41	0.72±0.45	84.12±3.6

3.7. Effect of LAB on chemical composition of Ayib

Proximate compositions of cheese (Ayib) produced with different starter cultures is shown in Table 10. The moisture content was significantly lower in Ayib produced with isolated starter cultures as compared to the control group (Table 6).

Table 6. The Effect of temperature and on pH of the Ayib

Ayib Samples	pH of Ayib produced at different temperature		
	70/45 ^{o}c/min	60/30 ^{o}c/min	50/45 ^{o}c/min
Ayib prepared with Lactobacillus spp.,only	4.40±0.32	4.64±0.35	4.84±0.33
Ayib prepared with Lactococcus spp.,only	4.08±0.33	4.34±0.35	4.34±0.32
Ayib prepared with both Lactobacillus spp$_{+}$ Lactococcus spp.	4.21±0.35	4.22±0.39	4.31±0.35
CONTROL	4.72±0.04	4.74±0.04	4.80±0.10

Lower moisture content implies better the quality because lower moisture content of cheese may lead to have longer shelf life. The average protein content of the three cheese samples did not differ significantly (Table 6). The protein content of the Ayib produced with isolated starter cultures was higher than the control. The total protein found in this study was higher than that obtained by Saria (2009). This may be due to the effect of starter culture and heat treatment which resulted in denaturation of whey protein and their retention in the curd. The high crude protein could also be attributed to the high levels of crude protein in the skim milk used for Ayib manufacture. The ash contents increased with the storage from the beginning till the end of the storage period. These results coincide the results that obtained by Elowni and Hamid (2008) and Kamal and Nagala (2009) who founded the ash contents of Sudanese white soft cheese increased during the storage period.

The fat content was observed to undergo a decrease of 1.9% and 2.78%, respectively; by the end of the storage period in the ayib fermentation made using the 1% and 5% inoculates. This decrease, which is sharper after 14 days of preservation, could be related to the growth of moulds, since those ones are the principal lipolytic agents in fermented milks (Tamime & Deeth, 1980). Formisano (1974) reported that the fat content of yoghurt decreased by 3.4% between culture and day 21 of storage. Other results demonstrated that the decreased in fat content was probably due to the

lipolytic activity of microorganisms on fat resulting in a leakage of some fat from crude into the pickling whey (Khalid (1991), Abbala (1992) Nofal *et al*. (1981) and (Nasur, 2001)). The increase of the fat content in the day 30 during the pickling could be attributed to the diminution of solids non-fat content due to the partial degradation of proteins and loss by solubility in whey (Nofa *et al*. (1981).

3.8. Effect of temperature on the pH values of the Ayib

The pH of Ayib produced with isolated starter cultures was lower than the control. No difference was observed in the pH of both isolates and the mixed starter culture. All types of cheese sample didn't show a great significant drop in all the samples reached a similar pH (4.3-4.6) towards the end of storage. The decrease in the pH of the fermented cereals (fermented products which are a mixture of starter culture a small proportion), the decrease in pH shows that the fermentation in a given type of product is taking place, with increased temperature of incubation agrees with work by Cooke *et al.* (1987) in which they evaluated acid production at 15°C, 25°C, 30°C and 37°C in yoghurt.

Conclusion

Strains of LAB were evaluated for their potential as starter culture based on the sensory attributes and final pH of the cultured milk. It is concluded that two species *Lactobacillu*s and *Lactococcus* the most dominant strains of LAB selected and used for Ayib production, and these isolates were identified having better flavor, texture and taste scores, with over all acceptance quality of the cheese. Texture profile analysis showed that there were changes in the quality of the cheese during storage for both the control and treatment samples. Microbial growth based on aerobic bacteria and coliform counts presented non-significant changes for all the period of evaluation of the treated cheese samples.

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