

JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCE SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF BIOLOGY

Microbiology of *Bukuri* and *Cabbage-Shameta* fermentation in ,East Wollega zone, Oromia region, Ethiopia

By: Biratu Chali

A thesis Submitted to the Department of Biology, College of Natural Sciences, Jimma University, In Partial Fulfillment of the Requirement for the Degree of Master of Science in Biology (Applied Microbiology).

June, 2014

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LIST OF ACRONYMS

AMB	aerobic mesophilic bacteria
CFU/ml	colony forming unit per milliliter
CV	coefficient of variation
EB	enterobacteriaceae
Н	hour
LAB	lactic acid bacteria
MRS	de Mann Rogosa Sharpe
PCA	Plate count agar
PDA	potato dextrose agar
ТА	Titratable acidity
TAPC	Total aerobic plate count

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ABSTRACT

Bukuri is a cereal based traditional fermented beverage prepared from locally available cereals (maize, barely, sorghum, millet, wheat, teff and malt flours: likewise, cabbage shameta is made from maize, barely and cabbage seeds (Brassica abissinicum) using traditional equipments in Nunu town, East Wollega zone. The objective of this study was to evaluate the microbiology of Bukuri and Cabbage-shameta fermentation. Seventy samples, comprising 35 each of Bukuri and cabbage-shameta, were collected from study area. Data related to the product description, raw materials and preparation techniques were collected using closed and open ended questionnaires. Samples were examined microbiologically for total aerobic mesophilic bacterial counts, lactic acid bacteria, Enterobacteriaceae, yeasts and moulds. Titratable acidity and pH of samples were also analyzed. Simple descriptive statistics and SPSS statistical soft ware (version 16) were employed for data analysis. The results revealed that Bukuri samples had total mean count of 6.84, 6.69, 6.72 and < 1 Log CFU/ml for Aerobic mesophilic bacteria, Lactic acid bacteria, Yeasts, Enterobacteriaceae and moulds respectively. The samples had a mean pH of 3.93 and TA of 3.23%. Similarly, cabbage-shameta samples had a total mean count of 6.66, 6.67, 0.53, 5.74 and 0.75 Log CFU/ml for Aerobic mesophilic bacteria, Lactic acid bacteria,, Enterobacteriaceae, Yeasts and Moulds respectively and mean pH of 3.96 and Titratable acidity of 7.42%. But during laboratory Bukuri fermentation from 0 hr to 72 hrs and cabbage-shameta from initial day to 14 days fermentation, the result had shown that Aerobic mesophilic bacteria generally reduced, Lactic acid bacteria and Yeasts increased, Enterobacteriaceae and moulds appeared initially and reduced to undetectable level then after throughout fermentation in both Moreover, the pH was reduced from 4.2 to 3.98, 5.21 to 3.92 and TA was samples. increased from 2.5 to 4.3%, 3.9 to 8.02% during laboratory bukuri and cabbage shameta fermentation respectively. By the end of 7 days of bukuri fermentation and 20 days of cabbage shameta fermentation, the pH was dropped to 3.52 and 3.79, respectively, the time when both products became too sour and unfit for consumption due too over acidification by LAB. It could be concluded that both Bukuri and cabbage –shameta are low-alcoholic, acidic products of relatively short shelf-life.

Keywords; Bukuri, Cabbage shameta, Ethiopian fermented food, traditional beverages

1. INTRODUCTION

In the world there are a large variety of fermented foods and beverages with traditional and cultural value. The diversity of such fermented products derives from the heterogeneity of traditions found in the world, cultural preference, different geographical areas where they are produced and the staple and/or by products used for fermentation (Marshall and Mejia, 2011).

Fermented foods and beverages harbor diverse microorganisms from the environment, which include mycelial molds, yeasts and bacteria, mostly lactic acid bacteria. Lactic acid bacteria are the most commonly used microorganisms for preservation of foods. Their importance is associated mainly with their safe metabolic activity while growing in foods utilizing available sugar for the production of organic acids and other metabolites. Their common occurrence in foods and feeds coupled with their long-lived use contributes to their natural acceptance as GRAS (Generally Recognized As Safe) for human consumption (Tamang and Kailasapathy, 2010).

However, there are many kinds of fermented foods in which the dominating processes and end products are contributed by a mixture of endogenous enzymes and other microorganisms like yeast and mould. Yeasts play vital roles in the production of many traditional fermented foods and beverages across the world that signifies the food culture of the regions and the community (Aidoo *et al.*, 2006).

Microorganisms transform the chemical constituents of raw materials during fermentation and enhance the nutritive value of the products, enrich bland diets with improved flavor and texture, preserve perishable foods, fortify products with essential amino acids, health-promoting bioactive compounds, vitamins and minerals, degrade undesirable compounds and antinutritive factors, impart antioxidant and antimicrobial properties, improve digestibility and stimulate probiotics functions(Tamang and Fleet ,2009).

Most of the ethnic fermented foods and beverages are produced by natural fermentation. Diversity within the species or strains of several functional genera of dominant microorganisms has created ethnic foods with different sensory characteristics (Tamang and Kailasapathy, 2010). A majority of traditional cereal based foods consumed in Africa are also processed by natural fermentation and these are particularly important as weaning foods for infants and as dietary staples for adults. Indigenous fermented foods are produced at the household level in a majority of African countries and as such represent an accessible option for increasing food security and through trade, a contribution to household income (FAO, 2012)

According to Rolle and Satin (2002) fermentation processes are believed to have been developed over the years by women, in order to preserve food for times of scarcity, to impart desirable flavor to foods and to reduce toxicity .Today, fermentation is still widely practiced as a household or village-level technology in many countries (Holzapfel, 2002). In Ethiopia, like in many developing countries, fermented food products and beverages constitute a major portion of peoples' diet. Ethiopia is a country rich in cultural diversity and hence, varieties of foods and beverages are processed and consumed among the various ethnic groups. The fermented products are, however, produced on fairly small scale and usually for local consumption (Ashenafi, 2006).

The reports of Abegaz *et al.*(2002) indicated that traditional fermented foods and beverages are those traditionally fermented products based on the skills of the household occupants by indigenous knowledge systems and is produced from a variety of locally available cereal ingredients using traditional techniques by the people of that area themselves. They became part of the cultural and traditional norm among the indigenous communities in rural and urban areas.

Indigenous Ethiopian fermented beverages include *tej* (Vogel and Gobezie 1983; Bahiru, 2000; Bahiru *et al.*, 2001), *tella* (Sahle and Gashe, 1991; Negussie, 2008), *borde* and *shamita* (Bacha *et al.*,1998), *areke*, traditional liquor (Desta, 1977), *Oromo Tella (Bukuri*) (Debebe, 2006) and *keribo* (Abwari, 2013).

Among the traditional Ethiopian fermented beverages, the fermentation processes and microbial dynamics during fermentation of '*Tella*' (Sahle and Gashe, 1991), 'Borde' (Bacha *et al.*, 1998) and '*Shamita*' (Bacha *et al.*, 1999) are described. Moreover, the

safety consideration of Ethiopian foods and beverages has shown the possibility of isolating some food-borne pathogens from some fermented products (Ashenafi, 2002).

Bukuri and *Cabbage-shameta* fermentations are indigenous traditionally fermented beverages produced and consumed in different parts of rural and urban areas of Nunu town, Nunu Kumba, East Wollega zone. *Bukuri* lies in between Gesho tella and borde. *Tella* is one of the Ethiopian traditional beverages, which is prepared from different ingredients. It is, by far, the most commonly consumed alcoholic beverage in Ethiopia (Debebe, 2006).

Depending on the type of cereal ingredients used to make, *tella* has different names: Amhara *tella*, Oromo *tella* and Gurage *tella* (Fite, *et al.*, 1991). Amhara *tella* has *Gesho* (*Rhamnus prenoids*) and concentrated. Gurage *tella* is delicately aromatized with a variety of spices. Oromo *tella* (*Bukuri*) has no *Gesho* (*Rhamnus prenoids*), and it is thick and taste sweet (Vogel and Gobezie, 1977). *Cabbage Shamita* fermentation is also important traditional fermented beverages. Taddesse *et al.* (2005) reported that *shameta* is produced by an over-night fermentation of certain cereals predominantly by LAB.

In Nunu town, Nunu kumba district, East Wollega zone, even though there is traditional fermentation of *Bukuri* and *Cabbage shameta*, there is no systematic research that has been done to determine the traditional product processes and microbial quality of *Bukuri* and *Cabbage* shameta. Hence, the present study was designed to evaluate the product processes and microbiological quality of *Bukuri* and *Cabbage-shameta* fermentation.

2. OBJECTIVES

2.1.1 General objective

To evaluate the microbiology of *Bukuri* and *Cabbage-shameta* fermentation in Nunu town, Nunu kumba district, East Wollega zone, Oromia region, Ethiopia.

2.1.1.1 Specific Objectives

- To document the traditional production processes and raw materials used during *Bukuri* and *Cabbage-shameta* fermentation.
- To assess the microbial load (quality) of *Bukuri* and *Cabbage-shameta* samples.
- To evaluate the microbial dynamics and changes in some physicochemical parameters (pH and TA) during the fermentation of *Bukuri* and *Cabbage-shameta* fermentation.

3. LITERATURE REVIEW

3.1 Fermentation

Traditional fermentation is a widely practiced ancient technology and fermented foods are an essential part of diets in all regions of the world (Ashenafi, 2002). Campbell-Platt (1994) claimed that around one-third of our food intake comprises fermented foods. Kwon (1994) estimated that around 20% of the total food consumed in the world is fermented foods. The types of foods processed using fermentation technology include beverages, dairy products, cereals and even meat products (Oyewole, 1997).

In Asia and Africa, and in some countries in Europe and Latin America, ethnic women more are actively involved in the preparation of foods using their native knowledge of food fermentation technology than men and also supplement culinary practices. There are about 5000 varieties of major and minor unlisted fermented foods and beverages in the world prepared and consumed by billions of people belonging to different communities and ethnicities. However, the consumption of some less known and uncommon ethnic, fermented foods is declining due to changes in lifestyle and the shift from cultural foods to commercial foodstuffs and fast foods (Tamang, 2010).

Steinkraus (1996) defined indigenous fermented foods as foods where microorganisms bring about some biochemical changes in the substrates during fermentation, such as the enrichment of the human diet through the development of a wide variety of flavors, aromas, and textures in foods; the preservation of foods through lactic acid, and alcoholic, acetic acid and alkaline fermentations ,the enrichment of food substrates biologically with proteins, essential amino acids, essential fatty acids and vitamins, the detoxification of undesirable compounds and the decrease in cooking times and fuel requirements.

3.1.1 African Traditional fermented food

Bahiru *et al.* (2001) informed that microbiology and biochemical properties of varieties of the indigenous African fermented food and beverages are available (table 1). These include Egyptian bouza, Tanzanian wanzuki, gongo, tembo-mnazi and gara, Nigerian palm-wine, Kenyan muratna and uragela, and South African kaffir beer.

Product name	Area of production	Substrate	Starter	
Burukutu	Ethiopia, and	Guinea corn and	Yeasts and lactic acid	
	Nigeria	cassava		
Pittokaffir beer	Northern Ghana	Guinea corn and		
		maize		
Busaa (maize	Nigeria and Ghana	Kaffir corn or	Moulds, yeast and	
beer)		maize	lactobacillus sp.	
Malawa beer	South Africa	Maize	Lactobacillus sp. and	
			yeast	
Zambian opaque	East Africa and	Maize	Yeasts and	
maize beer	Uganda		lactobacillus spp.	
Merissa	Zambia	Sorghum	Candida krusi	
Seketeh	Sudan	Sorghum	Yeast	
Bouza	Nigeria	Maize	Lactic acidbacteria and	
			acetic bacteria	
kishk	Egypt	Sorghum ,wheat	Lactobacillus spp.	
		and milk	yeasts and bacillus spp.	
Tella	Ethiopia	Barely malt, maize	Lactic acid bacteria,	
		,sorghum, millet	yeasts moulds and	
			bacillus spp.	

Table 1. Traditional fermentation of food and beverages from cereals in Africa (Marshall and Mejia, 2011)

A majority of traditional cereal based foods consumed in Africa are processed by natural fermentation and these are particularly important as weaning foods for infants and as dietary staples for adults. Indigenous fermented foods are produced at the household level in a majority of African countries, and as such represent an accessible option for increasing food security and through trade, a contribution to household income. It is however expected that increasing industrialization and urbanization trends in these countries may necessitate some scaling up of production of fermented foods of consistent quality (FAO, 1999)

3.1.2 Nutritional values and safety of fermented food

Fermentation processes enhance the nutritional quality of raw ingredient by improving the digestibility of nutrients and inactivating anti-nutritional factors (Chelule, *et al.*, 2010). They also improve acceptability of the food by destroying undesirable flavors of the raw ingredients (Steinkraus, 1996). Lowering the pH of food products through fermentation is a form of food preservation. This is a self-limiting process in that further reduction of pH may be unfavorable to the producing organisms. Therefore, fermentation technology is of great importance in ensuring food safety, preservation and food flavouring (Nout, 1997).

Traditional fermentation is a form of food processing, where microbes, for example, lactic acid bacteria (LAB) are utilized. The bacteria use food as a substrate for their propagation. This is a form of food preservation technology, used from ancient times. Over the years, it became part of the cultural and traditional norm among the indigenous communities in most developing countries, especially in Africa. The rural folk have come to prefer fermented over the unfermented foods because of their pleasant taste, texture and color. This popularity has made fermented foods one of the main dietary components of the developing world (Mosha and Vicent, 2004).

3.1. 3 Commercialization of fermented food and beverages

Several types of foods are traditionally fermented and this contributes substantially to the daily diets of rural communities. These indigenous foods are locally prepared in small scale, in the village homes; and their quality depends on the skills of the household occupants, as inherited over the years. These include alcoholic and non-alcoholic beverages, which are mainly cereal-based. Moreover, alcoholic traditional maize-based beverages have been produced at home-level and at a commercial scale (Zulu *et al.*, 1997).

3.1.4 Microbiology of fermented food

Fermented foods, a part of important food eco-system, harness a microbial diversity and converse the functional microbiota in the environment (Tamang, 2000). Filamentous moulds, yeasts and bacteria constitute the microbiota in indigenous fermented foods and

beverages, which are present in or on the ingredients, utensils or environment and are selected through adaptation to the substrate (Tamang, 1998; Antony and Chandra, 1997).

Thus, fermentation products in food substrates are based on the microorganisms involved in the fermentation. Some of the compounds formed during fermentation include organic acids (e.g., palmitic, pyruvic, lactic, acetic, propionic and butyric acids), alcohols (mainly ethanol) aldehydes and ketones (acetaldehyde, acetoin, 2-methyl butanol) (Campbell-Platt, 1994). The bacteria are not harmful to the consumer and have enzymes such as proteases, amylases and lipases that hydrolyze food complexes into simple nontoxic products with desirable textures, aroma that makes them palatable for consumption (Steinkraus, 1997).In foods and beverages LAB are the dominant microorganisms and therefore, lactic acid fermentation is considered as the major contributor to the beneficial characteristics observed in fermented foods (Oyewole, 1997).

3.1. 5 Significance of fermentation

3.1. 5.1 Flavour enhancements

Fermentation makes the food palatable by enhancing its aroma and flavor. These organoleptic properties make fermented food more popular than the unfermented one in terms of consumer acceptance (Blandino, *et al.*, 2003). Thus, fermentation is unique in that it modifies the unfermented food in diverse ways, resulting in new sensory properties in the fermented product (Leroy and De Vuyst, 2004). However, not all bacteria and moulds are beneficial in enhancing food flavor. In some instances, they may cause food spoilage since their enzymes may lead to generation of fermenting nutritional quality. A number of foods especially cereals are poor in nutritional value, and they constitute the main staple diet of the low income populations. However, LAB fermentation has been shown to improve the nutritional value and digestibility of these foods (Nout, 2009).

The acidic nature of the fermentation products enhances the activity of microbial enzymes at a temperature range of 22-25 °C. The enzymes, which include amylases, proteases, phytases and lipases, modify the primary food products through hydrolysis of polysaccharides, proteins, phytates and lipids respectively. Thus, in addition to enhancing

the activity of enzymes, LAB fermentation also reduces the levels of antinutrients such as phytic acid and tannins in food leading to increased bioavailability of minerals such as iron, protein and simple sugars (Chelule *et al.*,2010).

3.1. 5.2 Preservative properties

The preservative activity of LAB has been observed in some fermented products such as cereals. The lowering the pH to below 4 through acid production, inhibits the growth of pathogenic microorganisms which can cause food spoilage, food poisoning and disease (Schnurer and Magnusson, 2005). For example, LAB have antifungal activities . By doing this, the shelf life of fermented food is prolonged. This is because the shelf overgrowth of desirable edible bacteria in food out competes the other non-desirable food spoilage bacteria. Thus LAB fermented foods have lactic acid as the main preservative since lactic acid bacterial growth is accompanied by the production of lactic and acetic acids with decrease in pH and increase in titratable acidity.

3.1. 5. 3 Detoxification

Foods are often contaminated with a number of toxins either naturally or through infestation by microorganisms such as moulds and bacteria. Certain moulds often produce secondary toxic metabolites called mycotoxins. These include fumonisins, ocratoxin A, zearalenone and aflatoxins (Sweeney and Dobson, 1998). Several methods are available for degrading toxins from contaminated food, for example, using alkaline ammonia treatment to remove mycotoxins from food.

However, these methods are harsh to food as they involve use of chemicals which are potentially harmful to health or may impair/reduce the nutritional value of food. Cooking food does not remove mycotoxins either, as most of them are heat-stable. Detoxification of mycotoxins in food through LAB fermentation has been demonstrated over the years (Schnurer and Magnusson, 2005). Using LAB fermentation for detoxification is more advantageous in that it is a milder method which preserves the nutritive value and flavor of decontaminated food (Bata and Lasztity, 1999).

LAB bacteria also produce fungal inhibitory metabolites. These are mainly organic acids, which include propionic, acetic and lactic acids (Schnurer and Magnusson, 2005). Thus, LAB are applied as a hurdle against non-acid tolerant bacteria, which are ecologically

eliminated from the medium due to their sensitivity to acidic environment. Also, fermentation has been demonstrated to be more effective in the removal of Gram negative than the Gram-positive bacteria, which are more resistant to fermentation processing. Moreover, LAB are also known to produce protein antimicrobial agents such as bacteriocins (Aymerich, *et al.*, 2000). Bacteriocins are peptides that elicit antimicrobial activity against food spoilage organisms and food borne pathogens but do not affect the producing organisms.

3.1.6 Traditional fermented foods of Ethiopia

Ethiopian villagers prepare a wide range of traditional fermented foods and beverages from different raw materials such as cereals, ensete (false banana), honey, milk, etc. These products, if properly exploited, could be of significant economic importance for the country. Most of the customs and rituals involving the Ethiopian traditional fermented foods and beverages are still prevailing today in urban areas, village communities and rural households (Abegaz *et al.*, 2002).

A wide range of cereal based traditional fermented foods and beverages are prepared in Ethiopia. Some of the known beverages are *tella*, *borde*, *shamita*, *Bukuri*, *cheka*, *korefe*, *keribo*, *Merissa*, etc. *Tella* is alcoholic, while the rest are considered to be low or nonalcoholic beverages (Abegaz, *et al.*, 2002).Therefore Ethiopia is one of the countries where a wide variety of traditional fermented beverages are prepared and consumed (Ashenafi, 2000).

3.2 Microbiology of Bukuri and Cabbage-shameta fermentation

3.2.1 Bukuri fermentation

Bukuri is a traditional fermented beverage which is the most popular among all people of Nunu town, East Wollega zone and consumed by them. The ingredients for the preparation of *Bukuri* are cereals like barely (*Hordeum vuldare*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), wheat (*Triticum sativum*), finger millet (*Eleusine coracana*) or teff (*Eragrostis teff*) and it is without gesho. People use *Bukuri* as meal replacement, on holidays, wedding ceremony, social works and also as sources of income of many households. The popularity of traditionally fermented *Bukuri* is more common among the religious groups because it is considered as low alcoholic beverages. It has poor keeping quality with shelf-life of not more than three or four days. Bukuri is low-cost product in all aspect as it is usually manufactured using locally available equipments, ingredients and presence of sufficient number of professionals for *Bukuri* fermentation.

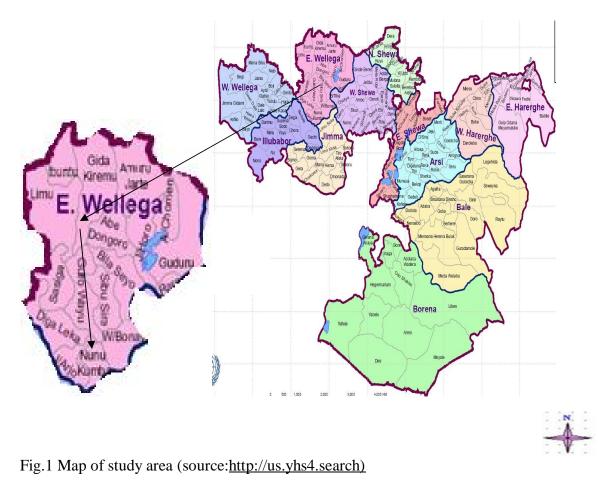
3.2.2 Cabbage-shameta fermentation

Cabbage-shameta is a traditionally fermented, low alcoholic beverage with a thick consistency mostly consumed by women in rural and urban areas of Nunu Kumba district, East Wollega zone. The ingredients for *Cabbage-shameta* fermentation are cereals like barley, maize, cabbage seeds and spices. *Cabbage-shameta* fermentation consumes time and energy and therefore it is not for sell, meal replacement, holidays and social works. The main purpose of *Cabbage-shameta* is for feeding of women who gave birth assuming that it facilitates the out flown of blood following child birth and it also improves quantity of breast milk. In *Cabbage-shameta* fermentation, the *Cabbage* seed is crushed using a mortar and pestle and soaked in water for about 5-9 days. After 5-9 days, the filtered liquid part is boiled with barely or maize flour and then cooled and spices are added and stored in a pot. The remaining solid parts are disposed. In *Cabbage-shameta* fermentation are locally available. The detailed flow charts of the production processes of both bukuri and cabbage shameta are given in the result section.

4. MATERIALS AND METHODS

4.1 Study area and period

The study was conducted in Nunu town, Nunu kumba district, East Wollega zone, Oromia region located 398 km West of Addis Ababa (Fig.1). Nunu kumba district lies within three climatic zones: *Dega, Weyna dega and Kola*. According to the static's of district agricultural office, the annual rain fall is 800-1000mm, annual temperature is 15-30°c and this district is known for its agricultural production of crops and rearing of animals. Nunu town is also known for its traditional foods and beverages including *Bunakala, Anchote, Bukuri*, *Cabbage-shameta, Gesho Tella* and others.



4.2 Sampling technique

A purposive sampling technique was used to address representative of *Bukuri* and *Cabbage-shameta* fermenters in Nunu town. The main aim of using purposive sampling technique was that *Bukuri* and *Cabbage-shameta* fermentation is done by

experienced women. Thus, to collect good information, experienced women with good knowledge of both *Bukuri and Cabbage-shameta* fermentation was involved. Therefore, the representative knowledgeable women could sufficiently explain the product process, equipments needed, raw materials, economic value and shelf life of *Bukuri* and *Cabbage-shameta* fermentation.

4.3 Sample Size Determination

From the pre- survey, a total of 260 women in Nunu town were engaged in the production of both Bukuri and *Cabbage-shameta*. So, the total sample size for both *Bukuri* and *Cabbage-shameta* fermentation was calculated using Cochran (1977) formula as follows:

$$n = \frac{n_0}{1 + \frac{n_0}{N}}$$
 $n_0 = \frac{Z_{\alpha/2}^2 p(1-p)}{d^2}$

Where

n = total sample size
d= margin of error
N = total number of the population
p= proportion of population
α= level of significance

d = 0.1
p = 0.5 and

$$\alpha$$
=0.05
 $n_0 = \frac{(1.96)^2 \times 0.5 \times 0.5}{(0.1)^2} = 96$

Considering the population correction factor into account the sample size should be:

$$n = \frac{96}{1 + \frac{96}{260}} = 70$$

4.4 Data collection

A total of 70 traditional fermented foods comprising 35 samples each of *Bukuri*, and *Cabbage-shameta* were collected randomly from knowledgeable women in Nunu town, East Wollega zone, Oromia region, Ethiopia between the months of December, 2013 – February 2014. A survey of indigenous processing methods and raw materials used for the preparation of *Bukuri* and *Cabbage-shameta* were carried out using open-ended and close ended questionnaires (Appendix I). The questionnaires had five categories: 1) indigenous processing techniques 2) types of equipment needed, 3) types and proportions of ingredients 4) economic importance and 5) shelf life of *Bukuri* and *Cabbage shameta*.

The brewers filled the questionnaires using their native language, Afan Oromo in the village or at their home (Appendix I). In addition, five each of *Bukuri* and *Cabbage-shameta* households was visited in order to observe the traditional preparation procedure and storage of the ready to consume *Bukuri* and *Cabbage shameta*. A total of 35 samples each of fermented *Bukuri* and *Cabbage-shameta* were collected and transported by ice box from Nunu-Nekemte to Jimma University, Biology Department, Research and Postgraduate Laboratory for microbial analysis.

4.5 Laboratory *Bukuri* and *Cabbage-shameta* preparation and Equipment

For convenience of handling and immediate analysis of samples, *Bukuri* and *Cabbage-shameta* were prepared in a laboratory using the traditional equipment and recipe at Jimma university post graduate laboratory. The appropriate equipments and raw materials were purchased and the cereals and malt were ground with cereal grinding mills locally. The cereal ingredients utilized for this investigation were maize (*Zea mays*), barley (*Hordeum vulgare*), wheat (*Triticum sativum*), sorghum (*Sorghum bicolor*), finger millet (*Eleusine coracana*), *Cabbage* seed and teff (*Eragrostis teff*). All of these cereals were utilized either as malts or unmalted ingredients. The main equipments were plastic jars , bowls, metal pot and sieve.

4.6 pH and Titratable acidity (TA)

The pH of *Bukuri* and *Cabbage-shameta* samples were measured using a digital pH meter after calibration at 25°C using buffers of pH 4, 7 and 10. TA was measured by titration of 5 ml sample with 0.1N NaOH using 0.1ml 0.5% phenolphthalein as indicator and then was calculated as percent. When a 5-milliliter sample and 0.1 normal sodium hydroxide was used, titratable acid was calculated using the following formula.

TA (%) = 0.15 X milliliters of sodium hydroxide used. The value of the constant (0.15) in the above equation was changed when a different size sample was used or when sodium hydroxide with normality other than 0.1 is used (Bolton, 1980).

4.7 Sample collection

A total of 70 ready to consume *Bukuri* and *Cabbage-shameta* samples comprising 35 samples each of *Bukuri* and *Cabbage-shameta* were collected from 35 different each *Bukuri and* Cabbage-*shameta* houses in Nunu town between the months of December, 2013 to February, 2014. Samples were collected and transported using ice box which is suitable for transport within one and half days. All the samples were brought to the laboratory and the microbial analysis were conducted. The samples were kept in the refrigerator at 4 ^oC until microbial analysis was conducted in Jimma University, Department of Biology and Applied Microbiology research and post graduate laboratory, found in main campus.

4.8 Microbiological methods

4.8.1 Sample preparation

After collection, *Bukuri* and *Cabbage-shameta* samples were analyzed for microbiological quality, including the enumeration of total aerobic mesophilic bacteria, Enterobacteriaceae, Lactic acid bacteria and total yeast and molds, for the determination of sanitary practices. For the procedure, 25ml each food samples were collected from *Bukuri* venders houses and *Cabbage-shameta* fermenters and mixed each with 225 ml sterile 0.1% Buffered peptone water and homogenized in a sterilized flasks for 30s using a shaker.

The homogenate was then serially diluted $(10^{-1} \cdot 10^{-5})$ and aliquots of 0.1 ml from appropriate dilutions was spread-plated in duplicate on pre-dried agar plates of plate count agar (PCA), Macckonkey agar (Oxoid), de Mann Rogosa Sharpe (MRS), and Potato Dextrose agar (PDA) for counts of different microbial groups.

Media was prepared according to the directions given by the manufacturers. The colonies were counted from plate containing microbial load between 30 and 300 colonies and expressed in Log colony forming units (Log CFU/ml).

4.8.2 Microbial Enumeration

4.8.2.1 Total aerobic plate count (TAPC)

For TAPC, a 0.1 ml aliquot spreaded Plate Count Agar (PCA) was incubated at 37°C for 48 hours (Trytinopoulou, *et al.*,2002). Then the total aerobic mesophilic count (AMC) was enumerated on PCA plates after incubation.

4.8.2.2 Enterobacteriaceae Count (EC)

To count the members of Enterobacteriaceae 0.1 ml of the aliquot, spread-plated on Mackonkey agar (Oxoid) was incubated at 37 0 C for 24 hours (Pons-Sanchez *et al.*, 2005). Then pink to red purple colonies were counted as member of a family of Enterobacteriaceae.

4.8.2.3 Lactic Acid Bacteria count

A volume of 0.1 ml of the aliquot was spread plated on de Mann Rogosa Sharpe (MRS) agar media and incubated at 30-32°C for 48-72 hours under anaerobic condition using GasPak jars (GasPak System, BBL) (Abegaz, 2007).

3.8.2.4 Total Yeast and Molds Counts

From appropriate dilutions, 0.1 ml aliquot was spread-plated on pre-solidified surfaces of Potato Dextrose agar plus 0.1g Chloramphenicol and incubated at 25-28 ^oC for 3-7 days for count of yeasts and mould (Abegaz, 2007). Smooth (non-hairy) colonies without

extension at periphery (margin) were counted as yeasts. Hairy colonies with extension at periphery were counted as molds.

4.9 Statistical Analysis

Data were organized and summarized using simple descriptive statistics and Microsoft office excels. Data analysis was computed using SPSS computer software (version 16.0).

4.10 Ethical Consideration

Study approval was obtained from department of Biology, Jimma University, and the Research and Ethical Review Board of College of Natural Sciences. Official permission was requested from study sites. The purpose of the study was explained to all respondents and concerned officials and informed consent for participation in the study was obtained prior to collecting the samples.

5. RESULTS

5.1 Socio-demographic Characteristics of the study population

A total of 70 women gave detailed information on raw materials used for *Bukuri and Cabbage-shameta* preparation procedures. All respondents were women consisting of maid or house wives (68.6%), civil servants (15.7%) and merchants (15.7%). Among these the proportions of Orthodox, Protestant and Muslim followers were 41.4%, 48.6% and 10%, respectively (Table.2).

Characteristics		Frequency	Percent
Sex	Female	70	100.0
Occupation	Civil servant	11	15.7
	Maid	48	68.6
	Merchant	11	15.7
Education status	Illiterate	11	15.7
	1-4	21	30.0
	5-8	19	27.1
	9-10	9	12.9
	11-12	1	1.4
	diploma	9	12.9
Religion	orthodox	29	41.4
	protestant	34	48.6
	Muslim	7	10.0
Age (year)	21-25	5	7.1
	26-30	13	18.5
	31-35	13	18.5
	36-40	20	28.5
	41-45	12	17.1
	46-50	7	10
	Total	70	100

Table.2 Socio-demographic characteristics of the study population of East Wollega,2014.

5.1.1 Description of the production processes of Bukuri

Bukuripreparation was the art and duty of females. The local producers of Bukurirelied on cereals as major ingredients including barely, maize, millet , sorghum ,wheatand teff. There was significant variation among respondents opinion concerning theproportion of the raw materials for preparation of Bukuri. The proportion has no standardthateachindividualfermentersuse.The materials used were pitcher or barrel, metal plates (mitad) and bowl. After thefermenters had decided on the proportions extent of raw ingredients and the type ofequipments needed, the quantity of each ingredient was measured before mixingaccording to the traditional recipe of Bukuri production. The base for Bukuri preparationwas malt which makes Bukuri fermentation quick. Malt was prepared mainly frombarely, millet and maize or by mixing either of the two.

For malt preparation, barley, millet or maize was first cleaned of broken kernels, chaff and extraneous materials. Then it was soaked with water for one day. Then after water was removed and barely was stored in a bowl for 3-5 days for germination. After 3-5 days, barely was germinated and became malt (**Fig.2**). Then, the malt was exposed to sun heat and dried and ground with local grinding mills.

The mean proportion of ingredients ,the respondents used in *Bukuri* preparation was 27.8 kg cereal powder ranging from 15-52kg for 5.1kg of malt ranging from 3-8kg in 69.14 liter of water ranging from 40 -120 L (**Fig 2**) .The container could be big, medium or small which was determined by the ratio of the ingredients used

Ingredients	Sample size	Mean ± SD	Minimum	Maximum	CV (%)
malt (kg)	35	5.11 ± 1.582	3	8	30.95
water (l)	35	69.14±29.44	40	120	42.58
cereal powder (kg)	35	27.80±10.16	15	52	36.54

Table .3 Mean weight of ingredients used for *Bukuri* preparation, East Wollega, Ethiopia 2014.

5.1.2 The traditional production process of *Bukuri*

The product process of *Bukuri* was so easy that people were widely consumed in their daily life. When *Bukuri* preparation was planned the type of ingredients, equipments and time of fermentation was selected. Then all the required cereals (barely, maize, millet and sorghum) were ready and cleaned out of the extraneous materials. Then it was roughly roasted on metal plates. After roasting the ingredients by mixing with each other, it was ground by locally available grinding mills. Then after grinding, it was made dough by mixing with water. The dough was fermented for about 5-7 days .After the end of fermentation 5-7 days, new flour was added to the fermented dough and mixed and stayed for about two hours before making to bread. Then the pitcher or the barrel was washed with the leaves of *Vernonia amyddalina* (girawa) in order to minimize the microorganisms.

The dough was made into bread and cooled and broken into smaller pieces and then together with malt and water was placed into pitcher or barrel by covering anaerobically.

The sequence for establishment of Bukuri fermentation into the pitcher was:

Malt \longrightarrow water \longrightarrow bread \longrightarrow malt. Finally on the second day of *Bukuri* fermentation the remained malt and water was added while mixing with the previous fermented *Bukuri* and was left for fermentation till ready for drinking on the third day (Fig.2)

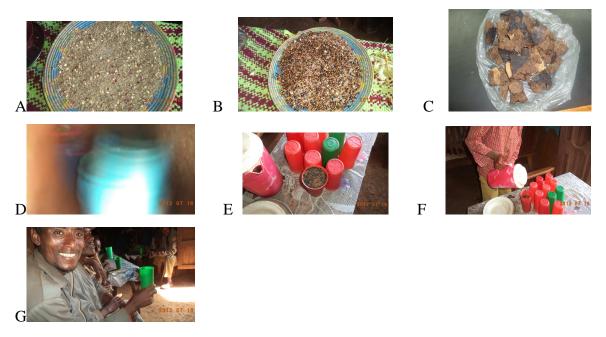


Fig. 2 Traditional *Bukuri* preparation in East Wollega, 2014.
A. malt B. Roasted cereals C. bread form D.plastic jar with *Bukuiri* E. ready *Bukuri* F. *Bukuri* poured to drink G. people drinking *Bukuri*

It was found that the traditional methods used for the preparation of *Bukuri* and fermentation period were similar in all households surveyed in the study area.

5.1.3 Laboratory Bukuri fermentation

The art of fermentation of traditional brewers is crucial for understanding every step used in the fermentation technology. Although the traditional brewers might not have scientific background, they could normally ensure a proper fermentation as a result of years of experience. Without knowledge of the art of traditional food fermentation, a scientist cannot provide a scientific explanation for the process and attempt to provide assistance in improvement of the technology. To this effect, *Bukuri* was prepared from a mixture of ingredients following the traditional recipe in Microbiology Laboratory for appropriate documentation of steps and procedures and immediate analysis of the product under study for some physicochemical parameters.

Based on information gathered during data collection, appropriate equipments and raw materials were purchased from local market in Jimma town. The main equipments and

ingredients suggested for *Bukuri* fermentation were plastic bucket, bowl, griddle of iron ('Mitad'), dish, maize, barely, millet and sorghum.

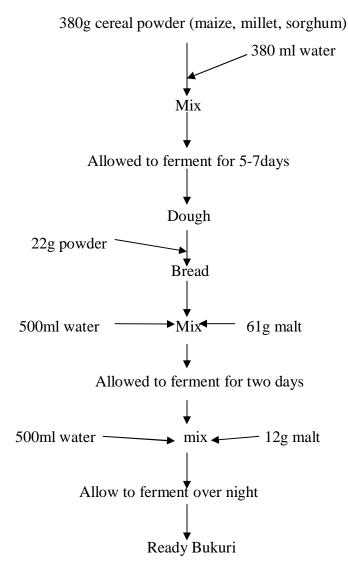


Fig. 3 Flow chart of Bukuri preparation process.

5.1.4 Quality and safety of Bukuri and cabbage shameta fermentation

Some of the advantages of traditional fermentation are that they are integrated into village life; commonly utilize locally produced raw materials, inexpensive and part of culture of local consumers. However, there is no scientific protocol for the production of traditionally fermented *Bukuri and cabbage-shameta*. During Fermentation day, the constituents and the quality of equipments were chosen according to the brewers

judgment. The quantity of water and substrate to be used for its fermentation was not regulated or standardized. The unpredictable processing environment, the hygiene of handlers, equipment and facilities were only dependent on washing with water alone. Therefore according to the respondents the shelf life of *Bukuri* was 3-5 days and 1 -2 weeks for *Cabbage-shameta* after ready to consume. The reason arose from the respondents for short shelf life of *Bukuri* were ,lack of hygiene and sanitation ,increased amount of malt and the nature of the short shelf .life of *Bukuri* itself. The spoilage of Cabbage-shameta was due to lack of hygiene and sanitation.

5.1. 5 Patterns of Bukuri and Cabbage-shameta consumption

It was found that all the respondents have been using *Bukuri* for household consumption, invite guests, during holidays, for social and group works, for source of income and weeding ceremony. It was considered as low- alcoholic beverage and was popular among all groups of people and was consumed daily. *Bukuri* differs from gesho tella in preparation procedure, alcoholic contents, short shelf life and lack of gesho. It was noted that *Cabbage-shameta* has low alcohol and consumed mostly by mothers after child delivery. This was because *Cabbage-shameta* preparation consumes time and energy.

5.1.6 Microbial load of *Bukuri* samples

In *Bukuri samples*, the minimum counts of Aerobic Mesophilic bacteria (AMB) was over 5 Log CFU/ml with the mean count of 6.84 Log CFU/ml and maximum counts of 7.74 Log CFU/ml. Similarly, the mean count of LAB in collected samples was 6.69 Log CFU/ml with maximum count of 7.74 Log CFU/ml (**Table 4**). The mean count of yeast in collected samples was 6.72 Log CFU/ml with minimum counts of 5.48 and maximum counts of 7.70 Log CFU/ml (Table 4). The counts of Enterobacteriaceae and Molds were below detectable level (<1 log CFU/ml) (**Table 4**).

LAB, AMB and yeasts were the commonly isolated microbial groups in *Bukuri* samples. There was statistically significant differences in the mean counts of LAB, AMB, EB, yeasts and moulds (CV>10) (**Table 4**).

Microbial groups	Sample size	Mean \pm SD	Minimum	Maximum	CV (%)
AMB	35	6.84 ± 0.80	5.48	7.74	11.69.
LAB	35	6.69 ± 0.73	5.05	7.74	10.91
EB	35	0.43 ± 1.25	.00	4.65	290
YEASTS	35	6.72 ± 069	5.48	7.70	10.26
MOULDS	35	0.50 ± 1.42	.00	4.61	284

Table.4 Mean counts (Log CFU/ml) of major microbial groups in Bukuri samples, EastWollega, Ethiopia, 2014.

Where: AMB = Aerobic Mesophilic Bacteria; LAB= Lactic acid bacteria; EB = Enterobacteriaceae;

5.1.7 pH and Titratable acidity of Bukuri Samples

Bukuri samples had average pH of 3.93 ± 0.15 ranging from 3.57-4.20 and mean titratable acidity (TA) of 3.23 ± 0.77 ranging from 2.40-4.50 (Table 5). There was no significant variation among *Bukuri* samples in pH (CV<10%), however, there was significant variation in TA values (CV>10%).

Table. 5 Physico-chemical change of Bukuri samples, East Wollega, Ethiopia, 2014.

parameters	Sample No	Mean \pm SD	Minimum	Maximum	CV (%)
ТА	35	3.23±0.77	2.40	4.50	23.8
рН	35	3.93±0.15	3.57	4.20	3.94

5.1.8 Microbial change during Laboratory Bukuri fermentation

At early stage of fermentation, the mean counts of yeasts were below detectable level until 24 hrs fermentation. Then after 24 hrs, it increased from 3.55 to 8.22 log CFU/ml at 72 hrs of fermentation. The counts of LAB increased for the first 24 h of fermentation from 3.47 to 5.34 log CFU/ml followed by exponentially increasing 30-36 hrs and then gradual increment thereafter to 7.65 log CFU/ml at 72 hrs fermentation (**Fig. 4**). Both EB and moulds remained below detectable level throughout fermentation with no significant

rise after 6 hrs with a total elimination at the end of 72 hrs fermentation. The counts of AMB, however, increased exponentially for the first 0 and 24 hrs of fermentation with gradual decline up to 72 hrs fermentation from 7.55 to 5.60 log CFU/ml (**Fig.4**).

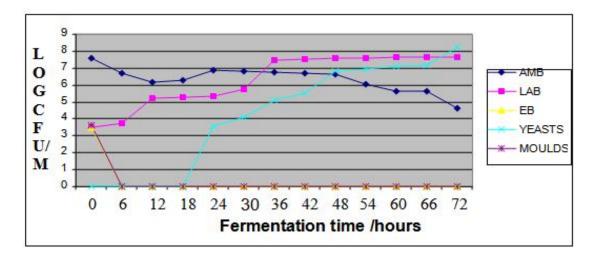


Fig.4 Microbial dynamics during laboratory fermentation of *Bukuri*, East Wollega, Ethiopia ,2014.

5.1. 9 physicochemical change of laboratory Bukuri fermentation

Changes in pH and TA during Laboratory fermentation of *Bukuri* are as shown in Fig. 6. The initial pH of unfermented Bukuri at 0 hr was around 4.28. The pH dropped to around 4.26 within the first 6 hrs of fermentation. The pH further dropped gradually to as low as 4.12 after 48 hrs of fermentation with maximum drop down to 3.98 in 72 hrs fermentation (Fig.5). Generally, titratable acidity increased from 2.50 to 2.68% during the first 6 h of fermentation. The reafter, the amount of lactic acid increased gradually up to about 3.40% during 48 hrs of fermentation. The amount of lactic acid produced reached a value of 4.30% at 72 hrs of fermentation (Fig.5).

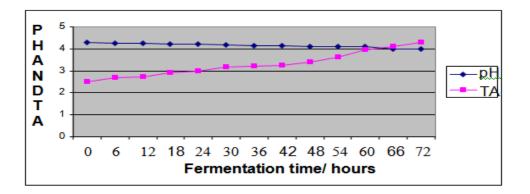


Fig. 5 physico chemical change during laboratory fermentation of *Bukuri*, East Wollega, Ethiopia, 2014.

5.2 Description of the production processes of Cabbage shameta.

5.2.1 Proportion of Ingredients for *Cabbage-shameta* preparation

Cabbage-shameta preparation was also the art and duty of females. The local producers of *Cabbage-shameta* relied on the ingredients of cereals like barely, maize, wheat , cabbage seed and spices. There was significant variation among respondents opinion concerning the proportion of the raw materials for preparation of *cabbage shameta*. The materials used were a pot or plastic jars or a bowl, a pestle, a mortar, sieve and traditional grinding stones.

After the fermenters had decided on the extent of raw ingredients and the type of equipments, for *Cabbage-shameta* preparation cabbage seed was selected, sun dried; partially ground with traditional grinding stones, ground deeply with pestle in a mortar ,it was soaked with water for 5-9 days (Fig.6). After 5-9 days the cabbage seed soaked was filtered and mixed with the cereal powder which were selected and cleaned from extraneous wastes and ground with local grinding mills. The powder was mixed with the cabbage seed filtrate and cooked for 20 minutes in the form of porridge by adding spices. Then it was cooled over night. After cooling it was stored in a pot or bowl covering tightly and followed to ferment anaerobically (Fig.6)



Fig.6 Traditional Cabbage-shameta preparation East Wollega, Ethiopia, 2014

A. cabbage seed selected B.cabbage seed roughly ground by traditional grinding stonesC. Deep grinding of cabbage seed in a mortar D. ground cabbage soaked in water E.cereal powder F. Cabbage-shameta pot G.Ready cabbage-shameta.

The mean proportion of ingredients, the respondents used ,in Cabbage-shameta preparation were 2.14 kg cereal powder ranging from 1.5kg-3kg for 0.05kg of cabbage seed and 1.3g spices in 22.5 liter of water ranging from 15 -30L (**Table.6**). The container could be big, medium or small which was determined by the ratio of the ingredients used.

Table.6 Proportions of Cabbage-shameta sample

Ingredients	Sample No.	Minimum	Maximum	Mean±S. D	CV (%)
cereal powder(kg)	35	1.50	3.00	$2.14\pm.39$	18.22
cabbage seed (kg)	35	.03	.07	.05 ±.01	20.00
spices (g)	35	1.00	2.00	$1.30 \pm .40$	30.76
water (1)	35	15.00	30.00	22.51±4.58	20.34

Cabbage-shameta was prepared from a mixture of ingredients following the traditional recipe in Microbiology Laboratory for appropriate documentation of steps and procedures and immediate analysis of the product under study for some physico-chemical parameters. (**Fig.7**). The main equipments and ingredients suggested for *Cabbage-shameta* fermentation were plastic jars, sieve, metal pot (biretdist), ground cabbage seed ,cereal powder or mixture of barely and maize and spices.

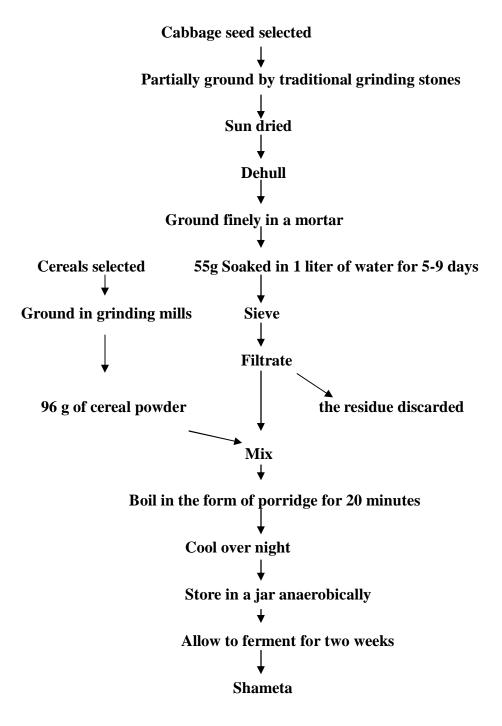


Fig.7 Flow chart for the preparation processes of Cabbage-shameta

5.2.2 Microbial load of Cabbage-shameta samples

The minimum counts of Aerobic Mesophilic bacteria (AMB) was 5.4 log CFU/ml with the mean count of 6.66 log CFU/ml and maximum counts of 7.7 log CFU/ml in *Cabbage-shameta* samples. Similarly, the mean count of LAB in collected samples was 6.67 log

CFU ml/ml with maximum count of 7.81 log CFU/ml (**Table 7**). The minimum counts of yeast was 1.01 log CFU/ml with the mean count of 5.74 log CFU/ml and maximum counts of 7.67 log CFU/ml. The counts of Enterobacteriaceae and Molds were below detectable level (< 1 log CFU/ml). LAB, AMB and yeasts were the commonly isolated microbial groups in Cabbage-shameta samples.

	Mean count (Log CFU/ml)				
Microbial groups	Sample No	Mean± S.D	Minimum	Maximum	CV (%)
LAB	35	6.67±.71	4.91	7.81	10.64
EB	35	.53±1.35	.00	4.56	254.71
Yeast	35	5.74±1.92	1.01	7.67	33.44
Moulds	35	.75±1.87	.00	5.54	249.33
AMB	35	6.66±.86	5.40	7.70	12.91

Table. 7 Mean microbial load (CFU/ml) of Cabbage-shameta samples, East wollega,Ethiopia, 2014

Where: LAB= Lactic acid bacteria; EB = Enterobacteriaceae; AMB = Aerobic Mesophilic Bacteria

5.2.3 pH and Titratable acidity of Cabbage-shameta Samples

Cabbage-shameta samples had mean pH of $3.96\pm.10$ and a mean titratable acidity (TA) of $7.42\pm.55$ (Table 10). There was no significant variation among Cabbage-shameta samples in pH and TA (CV<10%).

Table. 8 TA and PH of Cabbage-shameta samples, East Wollega, Ethiopia, 2014

Parameters	Sample No	Mean \pm ST.D	Minimum	Maximum	CV%
ТА	35	$7.42 \pm .55$	5.85	8.25	7.41
рН	35	3.96 ±.10	3.81	4.21	2.52

Where; TA= Titratable acidity

5.2.4 Microbial change during Laboratory *Cabbage-shameta* fermentation

At early stage of *Cabbage-shameta* fermentation, the counts of yeasts were below detectable level until second day of fermentation which was 3.70 Log CFU/ml and increased to 8.40 Log CFU/ml during twelve days of fermentation but decreased to 7.2 Log CFU/ml when *Cabbage shameta* was assumed to be ready to consume. The counts of LAB were also below detectable level during 0 hrs fermentation and were 5.40 Log CFU/ml on the second day and also increased gradually to 7.2 Log CFU/ml during tenth day fermentation and finally reached 9.3 log CFU in 14 day fermentation when *Cabbage-shameta* was ready to consume (**fig 8**). The counts of AMB, at start of fermentation were 5.4 log CFU/ml however, increased exponentially to 7.5 Log CFU/ml for the second days of fermentation with gradual decline up to 5 Log CFU/ml on fourteenth day of fermentation (**fig.8**). But EB and moulds were appeared <1 Log CFU/ml at initial and totally undetected throughout the fermentation day.

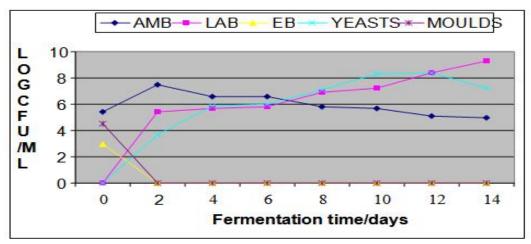


Fig.8 Microbial change during laboratory Cabbage-shameta fermentation, East Wollega, Ethiopia, 2014.

5.2.5 Physicochemical change during laboratory Cabbage-shameta

fermentation

Changes in pH and TA during Laboratory *Cabbage-shameta* fermentation is shown in (Fig. 9). The initial pH of unfermented at 0 h was around 5.21. The pH dropped slowly

from 5.21 to around 5.20 within two days of fermentation. The pH further dropped gradually to as low as 4.27 after 6 days of fermentation with maximum drop down to 3.92 in 14 days of fermentation (Fig. 9). Generally, titratable acidity increased from 3.9 to 4.2% during the first 2 days of fermentation. Thereafter, the amount of lactic acid increased gradually up to about 6.3% during 6 days of fermentation. The amount of lactic acid acid produced reached a value of 8.02% at 14 days of cabbage shameta fermentation (Fig. 11).

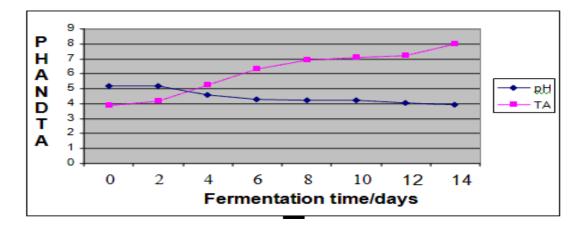


Fig.9 Change in pH and TA during fermentation of cabbage –Shameta fermentation, Jimma, Ethiopia, 2014

5.2.6 Spoilage of Bukuri and cabbage shameta fermentation

Bukuri is considered to be ready for consumption when it is bubbling. It is retailed at market place, or at vendors' house. According to the consumers and brewers, the most important sensory properties of a good quality *bukuri* are described as having a refreshing aroma, turbidity, sweet taste and a fairly smooth texture. This was at which LAB increased to 7 Log CFU/ml and yeasts about 8 Log CFU/ml at 72 hrs fermentation. When the active fermentation (bubbling) slows down, this is a signal of quality deterioration and after a while the product becomes sour and alcoholic as LAB were increasing to 8 log CFU/ml and yeasts 9 Log CFU/ml with reducing pH to 3.81.By the end of 7 days bukuri fermentation LAB increased to 9 log CFU/ml when the pH was dropped to 3.52 with rising of TA to 5% but yeasts were reduced to 4 log CFU/ml.

Similarly *cabbage shameta* is considered to be ready for consumption when it is mildly sour, having a refreshing aroma, turbidity, thick consistency and smooth texture. This was at which LAB increased to 9 Log CFU/ml and yeasts about 7 log CFU/ml at 14 days fermentation. By the end of 20 days fermentation LAB increased to about 9.5 log CFU/ml when the pH was dropped to 3.79 with rising of TA to 10.05% but yeasts were reduced to 6 Log CFU/ml. Finally both products became too sour and burning taste and unfit for consumption that might be due to metabolic products of LAB.

6. DISCUSSION

Indigenous food fermentation is one of the oldest 'food biotechnological processes dependent on the biological activity of microorganisms (Ross *et al.*, 2002), from which development of fermented foods are achieved in the cultural history of human being (Geisen and Holzapfel, 1996). *Bukuri* and *Cabbage-shameta* are one of these traditional fermented food and beverages of Ethiopia in Eastern Wollega zone. During the process locally available ingredient(s) are converted biochemically and organoleptically into upgraded edible products like other fermented foods (Campbell-Platt 1994; Steinkraus, 1996).

The socio demographic data of the current study indicates that *Bukuri* and *Cabbage-shameta* preparation are the duty and art of women in order to obtain nutritional quality, preserve food for longer time and collect income for their own family. This is similar with the reports of Tamang (2001) that indicated the culturally acceptable inexpensive foods provide a basic diet as side-dish, alcoholic and non-alcoholic beverages, which enhance nutritional quality, palatability, wholesomeness of the product with acceptable flavour and texture. So that women using their traditional knowledge of food fermentation technology usually prepare fermented foods, alcoholic and non-alcoholic beverages. Therefore *Bukuri* and *Cabbage-shameta* preparation are similar to the review of Ashenafi (2006) that other fermented foods of the world, are produced by using various manufacturing techniques, raw materials and microorganisms.

Bukuri is a traditional, low-alcoholic, dark brown colored, taste sweat while Cabbageshameta is low alcoholic ,brown colored with thick consistency and slightly sour fermented beverages commonly consumed in rural and urban areas of East Wollega zone, with some similarity to cereal based beverages of *koko* of Gahana (Lei *et al.*, 2006), *kanunzaki* of North Nigeria (Efiuvwevwere and Akona, 1995), *Mahewu* of south Africa (Sanni , 1993), *Bushera* of Western Uganda (Mukisa *et al.*, 2010) and *Motoho* of India (Gadaga *et al.*,2013).

Bukuri is produced by three days fermentation the same to Ogi of west Africa from maize, millet, sorghum and malt (Haard *et al.*, 1999). But Cabbage-shameta is produced

by fourteen days gradual fermentation of cereals (barley, or maize, cabbage seed) and spices predominantly by activities of LAB unlike the night fermentation of shameta (Bacha *et al.*, 1999; Ashenafi, 2006).

Both *Bukuri* and *Cabbage-shameta* is a multipurpose product, which are socio-culturally bound especially with the people of East wollega zone. Both are often a low-cost meal replacement for many poor people in the surrounding. The traditional recipe for both fermentation seems to be a very important way for the indigenous people to utilize a wide variety of crops. The cereal ingredients used vary from place to place in the communities that prepare and consume both.

Although it appears that each housewife has her own preference of ingredients, the overall technology of traditional preparation was found to be similar in the study area. The consumers' preference for preparation of both from blended cereals might be due to the organoleptic properties of the resulting product. According to Steinkraus (1996), fermentation can enrich food substrates biologically with protein, essential amino acids, essential fatty acids and vitamins; enhance the diet through a diversity of flavors, aromas and textures and decrease cooking times. Thus for the villagers, fermentation of *Bukuri* and *Cabbage-shameta* could be considered as an economical means of utilizing a variety of cereal crops.

In this study, it was found that Bukuri preparation requires about three days, unlike four days fermentation of *borde* reported by Ashenafi and Mehari (1995) in Awassa and overnight fermentation of *shameta* (Bacha *et al.*, 1999) in Addis Ababa. The processes involved in *Bukuri* production do not need starter culture (tinsis), unkuro and gesho. So that it is easier process and shorter fermentation time than *borde* and gesho *tella* (Ashenafi, 2006). But more complex than those of similar traditional fermented beverages such as Zimbabwean *mangisi* (Zvauya *et al.*, 1997), Nigerian *obiolor* (Achi, 1990), Sudanese *hulu-mur* (Mahgoub *et al.*, 1999), Turkish *boza* (Hancioglu and Karapinar, 1997), Tanzanian *togwa* (Mugula, 2001) and Ugandan *bushera* (Muyanja, 2001).

Unlike other Ethiopian traditional fermented food and beverages such as *borde, shameta* and *keribo* (Abwari, 2013), *Cabbage-shameta* fermentation has prolonged fermentation time for more than a week similar to Ethopian *tella* 10-12 fermentation day (Safaye,2011),Tej 8-20 fermentation day (Bahiru *et al.*,2006), Japanese *sake* 3 week fermentation (Matsushita *et al.*,2009), Indian *Bhaat jaanr* 7-8 fermentation day (Tamang ,2010), Indian *kodoko jaanr* 5-7 fermentation day (Tamang, 2005) and Indian *mana* 7 days fermentation(Tamang, 2010).

The raw materials required for *Bukuri* and *Cabbage-shameta* is roughly cooked before fermentation. This was reported by (Zvauya *et al.*, 1997) that as cooking temperature increased, amylase activity was also increased for the first 20 minutes and simultaneously free reducing sugars increased during production of *masvusvu* and *mangisi*. This is related with cooking of the ingredients that could gelatinize the starch and make it more easily available to create a more conducive environment for microbial growth during *Bukuri and Cabbage-shameta* fermentation.

Bukuri and Cabbage-shameta brewers also believe that the fermentation pot is not satisfactory for fermentation until it has been used several times. This is an indication that microorganisms are retained in the pot (Sahle and Gashe, 1991; Bacha, 1997) from the previous batch of fermentation. Several researchers (Johansson *et al.*, 1995; Damelin *et al.*, 1995; Bacha, 1997) have indicated that the microorganisms involved in the natural fermentation of cereals are essentially the micro flora of the raw materials and equipment. Thus, spontaneous fermentation of *Bukuri* could be initiated by a variety of microorganisms from the malt, fermentation equipment, blending water, brewing personnel and mixing utensils. Microorganisms from the malt and the fermentation equipment are probably more important due to their adaptation to the raw material of *Bukuri*.

Bukuri brewers prefer to use malt from more than one type of cereal. The report of Abegaz *et al.* (2002) indicated that the mixture of malt may have a wider array of endogenous enzymes that would achieve greater degradation of cereal starches. This creates a rich and complex growth medium for a variety of microorganisms initiating the spontaneous fermentation. After malt has been introduced to the fermentation pot, there is

no heat-treatment that would inactivate the malt amylases or the microorganisms present in the mash.

In case of *Cabbage-shameta* fermentation malt is not the ingredient for preparation like that of shameta (Ashenafi, 2006). All the ingredients including the soaked cabbage seed, cereal powder and spices pass through cooking. This cooked form of shameta porridge is cooled through night before stored and sealed in a pot or plastic container. Therefore the source of micro organisms expected for Cabbage-shameta fermentation was from heat resistance micro organisms, from the environment during cooling, personnel during preparation and the equipments like that of shameta (Bacha *et al.*, 1999).

In *Bukuri and Cabbage-shameta* fermentation ,high count of LAB could account for acidification of the product with extension of fermentation periods .This was reported by several researchers that LAB and yeasts have been involved in the natural fermentation of many traditional Ethiopian fermented foods and beverages (Bahiru *et al.*, 2006) and also in most of the African indigenous fermented foods and beverages (Nout, 1991; Halm *et al.*, 1993; Hounhouigan *et al.*, 1993; Steinkraus, 1996; Olasupo *et al.*, 1997; Nago *et al.*, 1998 and Kunene *et al.*, 2000).

According to (Gobbetti *et al.*, 1994; Stolz *et al.*, 1995 and Gobbetti and Corsetti, 1997) LAB and yeast enable the utilization of substances that are otherwise non fermentable (for example starch) and thus increasing the microbial adaptability to complex food ecosystems. In such processes that use malt to hydrolyze the cereal starch, an alcoholic fermentation develops spontaneously due to yeasts (mainly *Saccharomyces cerevisiae*) associated with lactic acid bacteria, which are responsible for the sour taste (Jespersen, 2005).These are applicable in *Bukuri and Cabbage-shameta* fermentation in which the sour taste and low alcohols are produced dominantly by LAB and yeasts respectively.

Although there are no microbiological standards set for the traditional fermented foods /beverages of Ethiopia, the mean counts of *Bukuri and Cabbage-shameta* samples were A MB 6.84 and 6.66, LAB 6.69 and 6.67, EB less than 1 in both, Yeasts 6.72 and 5.74 Log CFU/ml and Moulds less than 1 Log CFU/ml respectively. Similar findings were also reported on microbial load of AMB and yeast by (Ashenafi, 1994) in Injera, (Bahiru,

2001) in Tej, (Saleh, 2013) in Egyptian traditional milk and shameta (Bacha, *et al.*, 1999).

The laboratory *Bukuri and Cabbage-shameta* were prepared according to the traditional methods to study microbial dynamics and changes in some physico chemical parameters during the fermentation. The pioneer AMB initiated *Bukuri* fermentation at 0 h to 6 hrs by their early leading rate of growth to 7.55 Log CFU/ml followed by the initial colonization 3.47 Log CFU/ml of LAB. The initial pH 4.28 fermentation at 0 h could explain the reason for growth of AMB while the lower pH 4.26 at 6 h fermentation began to inhibit their growth. The LAB initially attained increasing with in each fermenting hours was responsible for a marked reduction of pH and increment in TA resulting in inhibition of most AMB in *Bukuri* fermentation. By the end of 72 hrs fermentation as the pH was slow down to 3.98 with the TA 4.30%, the count of AMB was reduced to 4.59 Log CFU/ml, in contrast the count of LAB was raised to 7.65 Log CFU/ml. This is similar with a microbiological study of Sobia, a fermented beverage of Saudi Arabia (Gassem, 2002) that reported 4-8 Log CFU/ml for both AMB and LAB at pH of 3.37-5.3.

In this study the yeasts appeared after 24 hrs of *Bukuri* fermentation when the pH was reduced to 4.2 and TA was increasing from 2.9 to 3.0 .This was directly related with the reports that has been suggested by (Nout, 1991) that the proliferation of yeasts in foods is favoured by the acidic environment created by LAB. The association of LAB and yeasts during fermentation may also contribute metabolites, which could impart taste and flavour to foods (Halm *et al.*, 1993; Brauman *et al.*, 1996 and Hansen, 1996). When pH was reducing through fermentation hrs by increasing % of TA the count of yeasts were increased from 3.55 Log CFU/ml at 24 hrs fermentation to the 8.22 Log CFU/ml at 72 hrs fermentation.

Cabbage-shameta fermentation was also prepared in laboratory according to the traditional recipe and microbial dynamics and changes in some physico- chemical parameters were studied for fourteen fermentation days. During initial day of Cabbage-shameta fermentation there was AMB with 5.40 Log CFU/ML that initiated the fermentation at 0 day to second day by their early leading rate of growth to 7.50 Log CFU/ML followed by the initial colonization of 5.40 Log CFU/ml of LAB when the

initial pH 5.21 at 0 day was reduced to 5.20 with raising of TA from 3.90 to 4.20%. The growth of LAB appeared during the second day of fermentation while it was a focal at which the reduction of AMB began by slow down of pH from 5.20 to 4.58. By the end of 14 days fermentation as the pH was lowered down from 5.21 to 3.92 with the raising of TA from 3.90 to 8.02%, the count of AMB throughout fermentation was reduced and finally to 5 Log CFU/ml, in contrast, the count of LAB was raised to 9.3 Log CFU/ml.

In *Cabbage-shameta* fermentation the yeasts appeared on the second day of fermentation at the same time with LAB when the pH was reduced from 5.21 to 5.20 and TA was increasing from 3.90 to 4.2% .This was directly related with the reports that has been suggested by (Nout, 1991) that the proliferation of yeasts in foods is favoured by the acidic environment created by LAB .As pH was reducing throughout fermentation day from 5.21 to 3.92 at the end of 14 days fermentation LAB were increasing while yeasts also were increased from 3.7 Log CFU/ML to 7.2 Log CFU/ml. Yeasts with 7 Log CFU/ml were also reported by (Tamang ,2010) in Indian Bhaat jaanr.

But EB and moulds were appeared during 0 h in both fermentation and automatically reduced to undetectable level after fermentation of 6 hrs and second day respectively. Thus, fermentation for 72 hrs and 14 days appeared to be a turning point for an accelerated reduction in number of AMB and maximum number of acid producing LAB and high counts of yeasts involved in both fermentation at which the pH was dropped to 3.98, 3.92 and TA was 4.3, 8.02% respectively .several studies were reported with some similarities with *Bukuri and cabbage shameta* fermentation incase of microbial and physico chemical changes (Bach*a et al.*, 1999) on Ethiopian shameta, (Bahiru *et al.*, 2006) on Ethiopian *Tej*, (Thapa, 2001) on Indian *Bhaat jaanr*,(Tamang, 2005) on Indian *kodoko jaanr*,(Tamang ,2010) on Indian *mana* and (Gassem,2002) on Saudi Arabian *sobia*.

After consumption day our study indicates that *Bukuri and cabbage shameta* products became too sour and burning taste by the end of 7 and 20 days fermentation ,LAB were increased ,pH was reduced to 3.52 and 3.79 respectively and unfit for consumption that is due to metabolic products of LAB. This was reported by researchers that lactic acid bacterial fermentation inhibits growth of spoilage and pathogenic microorganisms by

producing organic acids, hydrogen peroxide, antibiotic like substances and lowering of pH rapidly to a point where competing microorganisms are no longer able to grow (Kingamko *et al.*, 1994: Tanasupawat and Komagata, 1995: Nyanzi and Jooste, 2012). This restricts the growth and survival of spoilage organisms and some pathogenic organisms.

Lactic acid bacteria involved in fermentation are able to produce hydrogen peroxide, but lack the true catalase to break down the hydrogen peroxide. The hydrogen peroxide can, therefore, accumulate and be inhibitory to some harmful bacteria and to the LAB themselves (Blandino *et al.*, 2003: Reddy *et al.*, 2008: Holzapfel , 2002).Therefore in our study extreme sourness and burning taste in longer fermentation of bukuri and cabbage shameta is the effect of lactic acid bacteria products. LAB as they were the cause of fermentation of bukuri and cabbage shameta they are also the cause of the product spoilage which finally leads to their own death.

In general, during production and sales, local producers must always keep their personal hygiene to discourage contamination. Sellers should also ensure that they do not expose the fermented products during display because this may predispose them to contamination. Improving the processing condition and upgrading traditionally fermented food production could improve the food in-security problems of the community. In order to produce the desired amount of traditional fermented food and beverages, it calls for optimization of the production processes and/or techniques. Hence, future studies should include the selection of most suitable strains for starter culture development that may be used to scale up the production of *Bukuri* and *Cabbage-shameta* from households- level to large scale production.

7. CONCLUSIONS

- *Bukuri* and *Cabbage-shameta* are traditional beverages consumed by all groups of people in Nunu town, East Wollega zone.
- *Bukuri* is a traditional, low-alcoholic, dark brown colored, taste sweat while *Cabbage-shameta* is low alcoholic ,brown colored product with thick consistency and slightly sour.
- Bukuri and Cabbage-shameta preparation is the art and duty of females.
- The advantage of *Bukuri* and *Cabbage-shameta* are that they are integrated into village life, commonly utilize locally produced raw materials, inexpensive and part of culture of local consumers.
- The shelf life of *Bukuri* is 3-5 days and 1 -2 weeks for *Cabbage-shameta* after ready to consume.
- *Bukuri* is used for several purposes: household consumption, invite guests, for holidays, for social and group works, for source of income, for weeding ceremony and *Cabbage-shameta* is used for feeding lactating mothers.
- Initial heat treatment of the raw materials creates a more conducive environment for microbial growth during *Bukuri and Cabbage-shameta* fermentation. Thus, spontaneous fermentation of *Bukuri* and Cabbage-shameta are initiated by a variety of microorganisms that involve AMB, LAB, EB, Yeasts and Moulds.
- LAB are responsible for a marked reduction of pH and increment in TA in the course of fermentation resulting in inhibition of most AMB, EB and Moulds to the undetectable level.
- In general, venders and local producers must always keep their personal hygiene to discourage contamination and improve the processing conditions.

8. RECOMMENDATION

- Lack of standardization in the methods used, the environment and the hygiene of the people that prepare *Bukuri* and *cabbage shameta*, will determine the quality of the product. Good personal hygiene should be practiced to complement the overall benefits of fermented *Bukuri* and *cabbage shameta*.
- The technology needs to be improved through research to ensure that technology is used to add value to such products, such as increased shelf-life, flavor and appealing packaging and labeling.
- Molecular characterization of the microorganisms associated with Bukuri and *Cabbage-shameta* production is needed
- Bukuri and Cabbage-shameta fermentations rely on spontaneous fermentation process as the fermentation is initiated and dominated by the natural inocula associated to the raw materials and containers used for fermentation. This makes the process inefficient, uncontrollable, and unpredictable. In a controlled fermentation, however, the fermentative microorganisms are defined and product quality is also predictable. Thus, for better quality, predictable product, and marketability of the two traditional fermented low alcohol beverages, it calls for piloting of the production process using defined starter cultures developed from the traditional fermentation and planning for scaling up of the production system in the future.
- The biochemical role(s) of microorganisms associated with *Bukuri* and *Cabbage-shameta* fermentations needs to be determined through further chemical analysis of products released by the microorganisms under controlled laboratory conditions.

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Appendix I

Jimma University Post Graduate Studies Department of Biology (Applied Microbiology) Reaserch questionnaries

Objectives of Questionnaires

Dear respondent, these **questionnaire** was designed for identification of product process and microbiology of **Bukuri** (Oromo tella) and *Cabbage-shameta*fermentation in Nunu town, Nunu kumba district,East Wollega zone, Oromia region, Ethiopia.

Instruction: I. please give answers briefly and clearly. II. Circle a letter of your choice where there are alternatives.

I. Personal information

A. Sex: M___F___

B. Occupation: student _____ government- worker ____ maid ____ Farmer _____

C. Education status; A. none B. 1-4 C. 5-8 D. 9-10 E. 11-12 F.

Diploma G. digirii and above

D. Religion: A. Ortodox B. protestant C. muslim

II. Bukuri fermentation

1. Do you know Bukuri Tella? Yes or No

2. What are the materials (furniture) needed for the preparation of Bukuri

?_____

3. What are raw materials (cereals) used for Bukuri

preparation?_____

4. What are cereals for making malt and ways of malting?_____

5. How many grams of powdered barely malt is required? For how much liters of water and grams of powdered grains?_____

6. What are ways or procedures of Bukuri preparation? Write in flow chart._____

7. How would you keep the quality and safety of Bukuri ?

8. Duration of Bukuri process from preparation to consumption?A.2 days B.3 days C,4 days

9. What is the potential of Bukuri spoilage after ready to consume? A, Spoiled after

1-2 days B. Spoiled after 3-6 days C. Spoiled after a week

10. What is the factor behind the spoilage?

A. lack of safety B. increasing amount of malt C. its limited shelf life

11. What is socioeconomic value of Bukuri fermentation?

A. family foodB .for holidaysC. for social worksD. for weedingceremoniesE .for source of incomeF. for allG .others (clarify)12. What is the difference of Bukurifrom gesho tella?

A. way of preparation B. Bukuri is low alcohol content C. shelf life D. Gesho E. mention if other

13. How is the alcoholism of Bukuri ? A. Has no alcohol B. has low alcohol C. has high alcohol

*Note! If you have additional comment on Bukuri fermentation please write down.

III. Cabbage-shameta fermentation

- 1. What are the materials (furniture) needed for *Cabbage-shameta* fermentation?
- 2. What are raw materials (cereals) used for Cabbage-shameta fermentation?

3. By estimating for one pot *Cabbage-shameta* fermentation in kilo girams /liters A.cereals required in kilogram_____B.water required in liters _____

C. Cabbage seed required in kilograms_____Spices in grams _____

4. What are ways (procedures) for Cabbage-shametapreparation?

5. What quality and hygiene is kept during Cabbage-shametafermentation?

6. How many days are required for *Cabbage-shameta* fermentation?(from preparation to consumption)_____

7.How many days are required for *Cabbage-shameta*consumption after it is already started consuming? A.1-2 days B. 3-5 days C. a week D. 2-4 weeks
8. Is it possible to make shameta with out using *Cabbage* seeds?
9. What is the use of *Cabbage* seed for shameta

fermentation?_____

10. What is the socio economic value of *Cabbage-shameta*fermentation?

A. for all family meal replacement B. for mothers during child birth C. for holidaysD. for social works E. For weeding ceremonies F. for source of income G. others11. How do you consume shameta ?(by eating or drinking)

Thank you for your cooperation!

Universiitii Jimmaa Kolleejjii Saayinsii Uumamaa, Muummee Baayolojii Sagantaa Barnoota Digrii 2ffaa Appilaayid Maaykiroo Baayolojii

Gaaffilee qorannoof Dhiyaatan Kaayyoo gaaffilee qorannoo kanaa

Kabajamoo! hirmaattotaa, gaaffileen kunnen kan dhiyaataniif akkaataa hojjechuu fi maaykiroo baayolojii farsoo Bukuri fi Shameta ija goommanaan hojjetamu Magaalaa Nuunnu, Aanaa Nuunnu Qumbaatti Wallaga Bahaa ,Naanno Oromiyaa ,Dhiya Itiyoophiyatti argamu qorachuufi dha.

Ajaja I ;Deebii keessan karaa gabaabaa fi ifaa ta'een kenna

II. Bakka filannoon jirutti qubeetti maruun deebii kessan kenna.

I. Odeeffannoo dhuunfaa

A. Saala : dhi____dha____

B. Gahee hojii : A. barataa B. hojjetaa mootummaa C. haadha warraa D. qonnaan bulaa

C.Haala barnootaa; A. hin baranne B. kutaa 1-4 C. kutaa 5-8 D. kutaa 9-10 kutaa 11-12 F. dipilooma G. digirii fi isaa ol

D. Amantaa; A. Ortodoksii B. protestantii C. musliima

II. Farsoo Bukuri naquu (hojjechuu) ilaalchisee

1. Farsoo Buqurii beektaa? Eyyee ykn Lakki

2. Meeshaleen ykn qodaan farsoo Bukuri naquuf barbachisan

maalfai?_____

3.Midhaan callaan farsoo Buqurii naquuf oolan maalfa'i?_____

4. Biqila farsoo Buqurii kan ta'uu danda'an maalfa'i? Akkaataa biqilli itti hojjetamu duraa duubaan tarressaaa?_____

5. Tilmaamaan hammi daakuu biqilaa Buqurii gaanii ykn barmela tokko naquuf barbaachisu kiilograama meeqa?Gaanii moo barmelatti fayyadamtu?

A. daakuu biqilaa,kilogiramii_____B.bishaan litrii_____C.daakuu midhaanii kilogiramii_____

6. Mee adeemsa farsoo buqurii naquu duraa duubaan tarreessaaa._____

7. Qulqullina farsoo buqurii eeguuf maal maaltu godhama?_____

8. Farsoon Buqurii naqamee guyyaa meeqatti dhugaatiif

gaha?_____

9.Dhugamuu jalqabee hoo hamma dhugaatii dhowwutti (alaa'utti) hammam

tura?_____

10. Alaa'uu farsoo Buquriitiif sababni maali jettanii yaaddu?

A.Qulqullina dhabuu B. daakuun biqilaa itti baaya'chuu C. tajaajilli farsoo Buqurii yeroo gabaaba qofaf ta'uu

11. Bu'aan farsoon Buqurii hawaasummaa kessatti qabu maalfa'i?

A.Dhugatii maatii	B .dhugaatii guyyaa ayyaanaa	C. hojii daboo fi gareef
D.guyyaa fuudhaa fi heer	rumaatiif E.galii argamsiisuuf	F .hundumaaf G. kan biraa
voo jiraate ibsaa		

12. Garaagarummaan farsoo Buqurii fi farsoo geeshoo maali?

A. adeemsa itti naqaman ykn hojjetaman B.macheessuudhaan buquriin gadaanaa dha

C.yeroo turtii tajaajilaa kennuu D. Gesho E.hundumaa F. kan biraa yoo jirate

III. Shaameta goommanaan naquu(hojjechuu) ilaalchisee

1. Meeshaleen ykn qodaan shameta goommanaa naquuf barbaachisan maalfa'i?_____

2. Midhaan callaan Shameta goommanaa hojjechuuf oolan maal

fa'i_____

3. Tilmaamaan shameta Okkotee(a pot) tokko naquuf; A. midhaan waligalatti

barbaachisu kilogirama ______ B. Goommana barbaachisukilogiraama_____

C. mi'eessitu barbaachisu giraamaan_____D. Bishaan barbaachisu litira_____ kan biraa yoojiraate_____

4. Me akkaataa shaametaan goommanaan hojjetamu duraa duubaan kaa'a?_____

5. Qulqullinnii fi eeggannon hojii shametaaf godhamu maalfa'i?

6. Shaametni gommanaan hojjetamu naqamee guyyaa meeqatti tajaajilaaf gaha?

7. Shaametni tajaajilaf gahee,guyyaa hammamiif turuu danda'a? A. Guyyaa 1-2

B. guyyaa 3-5 C. torban tokko D. torban 2-4

8. Ija goommanaa malee shameta hojjechuun ni danada'amaa? Eyye ykn Lakki

9. Shameta hojjechuuf faayidaan ija goommanaa maali?_____

10. Hawaasummaa kessatti faayidaan shametaa goommanaa maalii?

A.Nyaata maatiif B. Nyaata haadholee yeroo da'umsaaf C.Guyyaa ayyaanaaf D. hojii daboo fi gareef E. guyyaa gaa'elaaf F. Galii argachuuf G. hundumaa

11. Shametni attamitti nyaatama ykn dhugama?_____

Guddaa galatoomaa!