

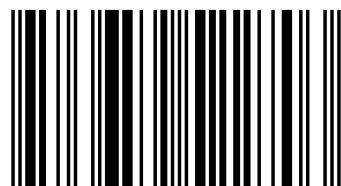
In Ethiopia, most of milk production and processing take place at small holder level where dairy infrastructure is not yet well developed. Very little is known about the effect of traditional ways of dairy product preservation methods that practiced by the smallholder dairy producers. This book therefore provides essential information on the effect of locally available spices on the shelf life and consumer acceptability of Ayib - an acid-heat coagulated cottage type soft cheese. The book should thus be useful to dairy producers, researchers , organizations, graduate students and public bodies.

The Case of Ginger and Garlic Powder



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978-3-659-52270-3

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# Effect of Spices powder on Quality of Ayib- Ethiopian Cottage Cheese

The case of Ginger(*Zingiber officinale*) and Garlic (*Allium sativum*) powder

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## **Impressum / Imprint**

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OmniScriptum GmbH & Co. KG

Heinrich-Böcking-Str. 6-8, 66121 Saarbrücken, Deutschland / Germany

Email: [info@lap-publishing.com](mailto:info@lap-publishing.com)

Herstellung: siehe letzte Seite /

Printed at: see last page

**ISBN: 978-3-659-52270-3**

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## **ACKNOWLEDGMENT**

I sincerely acknowledge the financial support of Adama Science and Technology University. The administrative, technical, material, financial and/or moral supports of Dr. Zelalem Yilma from (EDDA), Dr. Eyassu Seifu from Haramaya University, Dr. Getenet Aseffa (EIAR), Biniyam Kassa, Zewdie Wondatir, Firew Kassa, Rahel Nebiyu, Aleganesh Tola, Alemitu Beyene and Kassahun Yemane from Holetta Agricultural Research Center, Biruk Hailu, Muluken Gofish and Emun from Haramaya University; and Endashaw Terefe from Adama Science and Technology University are highly appreciated.

## LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Associations of Official Analytical Chemists
AMBC	Aerobic Mesophilic Bacteria Count
ARN	Acidic Ribonucleic
CC	Coliform Count
CEC	Commission of the European Communities
CSA	Central Statistical Authority
<sup>0</sup> C	Degree Centigrade
CFU/g	Colony Forming Units per gram
DNA	Deoxyribonucleic Acid
FDA	Food and Drug Administration
GLM	General Linear Model
IFST	Institute of Food Science and Technology
KDA	Kilodalton
m. a. s. l	meters above sea level
RNA	Ribonucleic Acid
SAS	Statistical Analysis System
USDA	United States Department of Agriculture
USFDA	United States of Food and Drug Administration
w/w	weight by weight
YMC	Yeast and Mould Count

## 1. INTRODUCTION

The bulk (98%) of the milk in Ethiopia is produced in rural areas by subsistent farmers where there are limited dairy infrastructure/facilities (Tsehay, 1998) and the dairy sector contributes to about 20-25% to the agricultural sector (Getachew and Gashaw, 2001). Milk is the most complete food product of animal origin providing essential nutrients in significant amounts than any other single food and contains fats, proteins, lactose, vitamins and minerals (Fox *et al.*, 2000).

On the other hand, the composition of milk makes it an ideal medium for the growth of both spoilage and pathogenic microorganism (O'Connor, 1994). However, special consideration is needed on the quality to supply safe milk and milk products to the consumer. In Ethiopia, there occurs seasonal variation in milk production that has implication in milk price. Prices lower during high production following more feed availability as a result of the rainy seasons. Moreover, milk losses up to 20-35% have been reported from milking to consumption due mainly to mishandling, uneconomical use or efficiency of low level of technology in preservation and conversion of milk (Getachew, 2003).

Processing milk into butter and cheese is an ideal vehicle for preserving the valuable nutrients present in milk. In Year 2010/11, about 45% of milk produced at national level was converted into butter, cheese and yoghurt (CSA, 2012). Through milk shed specific, processing milk into more shelf stable products such as butter not only increases income but also facilitates easy delivery to the market. According to an earlier study, the sale of milk products especially butter and *Ayib* (Ethiopian Cottage Cheese) in the highlands of Ethiopia provides 28% increase in rural household income (Von Massow, 1998).

There is a growing interest in traditional dairy products, especially cheeses manufactured at the farm level due in part to the uniqueness of such food and the difficulties in mimicking them on an industrial scale. In the global market, these traditionally produced cheeses are highly appreciated and are considered to be of premium value due to their flavor characteristics, which are not found in a number of industrially produced cheese varieties (Garabal, 2007).

*Ayib* is a popular fermented milk product consumed by the various ethnic groups in Ethiopia. It is made from sour milk after the butter is removed by churning. *Ayib* comprises about 79% water, 15% protein, 2% fat, 1% ash and 3% soluble milk constituents (Mogessie, 1992). It is consumed as traditional side dishes with *Kitifo* (minced meat) and *Doro wet* (chicken sauce).

The microbial qualities of *Ayib* manufactured at the different places and different producers differ, this is assumed to be the result of variations in production, processing and preservation practices followed at different stages. There is no as such a standard method of processing and preservation of milk products in Ethiopia based on local production conditions (Zelalem and Faye, 2006).

Earlier works reported research results that improved the quality and yield of *Ayib*. Mogessie (1990b), for instance, indicated that heating the curd at 70°C during *Ayib* making improved its microbial quality. Zelalem *et al.* (2007), on the other hand, indicated yield expressed by weight of *Ayib* recovered to increase with increasing the length of time the curd stayed in the whey before separation. As reported by the same authors, 1266, 1475 and 1776g of *Ayib* was obtained per an average of 9.2 liters of sour defatted milk after keeping the *Ayib* in the whey for 1hr, 2hr and overnight,

respectively. Extending the shelf life of *Ayib* for 3.35, 4.63, 3.94 days by seasoning with rue juice, garlic juice and their mixture, respectively was also reported (Binyam, 2008).

*Ayib* has the shelf life of no more than two days at ambient temperature of about 30°C, while at 4°C its shelf life is about seven days (O'Connor, 1993). Compare with other semi-hard and hard cheeses, *Ayib* has a short shelf life due to its high moisture content. Thus, application of appropriate preservation techniques is required in order to retard microbial growth and extend the keeping quality of *Ayib*.

The safety of milk products with respect to food-borne diseases is of great concern around the world. This is especially true in developing countries where production of milk and various milk products takes place under rather unsanitary and poor production conditions (Mogessie, 1990). However, studies showed that smallholder milk producers in Ethiopia have their own traditional way of improving the quality of milk and milk products. These include: smoking milk containers (Mogessie and Fekadu, 1993; Aleganesh, 2002; Lemma, 2004; Helen, 2007); washing the utensil with water and leaves of shrubs (Aleganesh, 2002; Lemma, 2004; Helen, 2007; Binyam, 2008; Yitaye *et al.*, 2009); putting *Ayib* in a sieve container and squeezing it at intervals for about 3 days until the water content is sufficiently drained and become dry enough; and seasoning with spices (Yitaye *et al.*, 2009). Seasoning *Ayib* with spices such as *Allium sativum* (Nech Shinkurt), *Ruta graveolens* (Tena adam), *Nigella sativa* (Tequr azmud), *Zinger officinale* (Zingibel), *Ocimum hardiens* (Koseret), *Cupisum spp.*(chillies), *Elettaria cardamoum* (Korerima), *Brassica nigra* (Mustard), *Ocimum spp.*(Basil), *Allium cepa* (Qey shinkurt), *Trachyspermum copticum* (Nech azmud), *Trigonella foenum* (Abish), *Ocimum bacilium* (Besobila) has been reported by some authors (Aleganesh, 2002; Binyam, 2008).



As indicated by Agatemor (2008), in the global food industry more priority is given to natural preservatives. This is due to the increase in occurrence of resistance in pathogenic strains against chemical preservatives. Levy (1997 cited by Souza *et al.*, 2005) reported that fifty years of increasing use of chemical antimicrobials have created a situation leading to ecological imbalance and enrichment of multiples multi-resistant pathogenic microorganisms.

According to Mishra and Behal (2010), natural products such as spices can be appropriate alternatives to chemical preservatives used in various food industries minimizing their possible side effects and simultaneously improve the shelf life of food products. Souza *et al.* (2005) also reported that spices and their derivatives could be suitable alternatives for inclusion in food preservation systems where they can act as main antimicrobial compounds. Antimicrobial activity of spices is dependent on several factors, which includes type, composition, processing and storage conditions of spices and microbial species and their level (Farag *et al.*, 1998).

In Ethiopia, most of the smallholder dairy producers and a substantial number of consumers do not have refrigerator and other modern preservation methods. It is, therefore, difficult to store milk and milk products for a long time. The use of natural plant preservatives, therefore, is considered an important and practical choice. Different types of plants are used to improve the flavor, color and quality of milk and milk products (Fekadu, 1994; Zelalem *et al.*, 2006; Helen, 2007; Binyam, 2008). The rhizomes of *Zingiber officinale* (Zingibel) and *Allium sativum* (Netch Shinkurt) are spices commonly used as additives in *Ayib* processing (Binyam, 2008; Yitaye *et al.*, 2009).

Stoilova *et al.* (2007) indicated that ginger is used as ingredient in making soups, as a spice in ginger bread, can be made into candy, as flavoring for cookies, crackers and cake and also as food preservative. Katzer (2005) reported that garlic is also consumed in various dishes, yoghurt, sauces, pickles and dried meat products and can locally paired with ginger to make stews and soups. According to Aruoma *et al.* (1997), both ginger and garlic have beneficial effects to human health because they exert antioxidant activity.

Ginger (*Zingiber officinale*) and garlic (*Allium sativum*) are reported to have strong antimicrobial properties. For instance, Belewu *et al.* (2005) showed that ginger extract treatment extends the shelf life of West African soft cheese for 15 days. Indu *et al.* (2006) also reported that garlic would increase the shelf life and decrease the possibilities of food poisoning and spoilage in processed foods. Adekalu *et al.* (2009) in their study on the antimicrobial and preservative activities of *Allium sativum* and *Eugenia aromatica* on fresh tomato puree reported that treatment with *A. sativum* at 4-5% preserved the tomato puree for 6 - 10 days, while the control went bad before 24 h. Gundogu *et al.* (2009) in their studied on the effect of garlic (*Allium sativum* L.) on some quality properties and shelf life of set and stirred yoghurt also reported that yoghurt treated with garlic can be consumed safely for 28 days, while the control can be consumed for only 7 days. However, there is no or very limited study or report on the effect of commonly used spices in general and ginger and garlic powder in particular on the microbial and sensory quality of *Ayib* in Ethiopia. The purpose of this study is, therefore, to determine the effect of ginger and garlic powders concentrations on the shelf life of *Ayib* and also to assess the effects of ginger and garlic powders mixture proportions on consumer acceptability of *Ayib*.

## 2. LITERATURE REVIEW

### 2.1. Production and Consumption of Milk and Milk Products in Ethiopia

Most of the milk production in Ethiopia comes from cattle (95.1%) and camel (4.9%) (CSA, 2011). The traditional milk production system, which is dominated by indigenous breeds, accounts for about 97% of the country's total annual milk production (Getachew, 2003). According to the report of Getachew and Gashaw (2001), 63% of the total milk production is produced by rural small-scale mixed farms in the highlands, 14.3% by small urban/peri-urban farms in the highlands, 22% by pastoral/agro-pastoral producers in the lowlands and less than 0.03% by large private and state farms. From the total annual milk production at country level, 85 percent is used for household consumption, about 7% is sold, only 0.3 % is used for wages in kind and the remaining 8% was used for other purposes such as production of edible and cosmetic butter and *Ayib* (CSA, 2010).

Milk is utilized in the production of at least 400 different fermented products all over the world (Willey *et al.*, 2008). Ethiopians consume milk either in fresh or fermented (sour) form. Traditional milk processing such as churning soured milk to make butter, dehydrating butter to make ghee and heating skimmed sour milk to make cottage cheese (*Ayib*) are common practice (Getachew and Gashaw, 2001). Though Ethiopia has a large cattle population, the per capita milk consumption is low estimated at 17 kg per head compared with the average figure (26 kg per head) estimated for Africa (Gebrewold *et al.*, 2000). The average expenditure on milk and milk products by Ethiopian households accounts for only 4% of the total household food budget (Staal *et al.*, 2008). In lowland areas, milk is consumed by all groups of the society, while in highlands the rural people mostly use milk for rearing calves and children with the

surplus being processed into more shelf stable fermented milk products mainly Ergo (Ethiopian naturally fermented milk), butter and *Ayib*. As reported by CSA (2010), of the total butter produced 61% is used for household consumption and 36% is sold. Most of the total Cheese produced (87%) is used for household consumption and the rest about 3% is used for other purposes.

The contribution of milk and milk products sales to the farming household income is significant. Fekadu and Abrahamsen (1994), for instance, indicated that the sale of milk and milk products contribute about 46% of the total household income and especially that of butter to be 20% of the rural household income in the Ethiopian highlands. Zelalem (1999) also reported that income generated from dairy operations on average accounted for 20 and 36% of the total household income around Holetta and Selale, respectively. As Zelalem *et al.* (2011) reported, more milk products especially butter (36.4%) and *Ayib* (10%) were sold as compared to milk (6.9%) in 2009/10.

In Ethiopia, a substantial proportion of the annual milk production at national level is wasted. As revealed by Getachew (2003), an estimated post harvest losses of milk and milk products from milking to consumption reached up to 40% due to factors. These include: contamination during milking and further handling coupled with storage time and temperature before consumption; deliberate adulteration of milk; substandard handling, transportation and distribution systems; inefficient processing technologies; inadequate fresh milk outlet; and spillage losses during milking. Tezera *et al.* (2005) also reported that annually, 14.2 million US dollars worth of the total value of annual milk is lost in Ethiopia.

## **2.2. Handling of milk and milk products**

Milk is relatively perishable and a high percentage of it is consumed in a relatively natural state, handling of milk and its products to preserve its natural and desired characteristics is very important (Duane and Cunningham, 1991). In areas where the climate is hot and humid, raw milk spoils easily during storage unless it is cooled or when possible treated with preservatives. However, these preservatives are not readily available in rural areas and cooling systems are not feasible because of lack of facilities (O'Mahony and Peters, 1987). In these areas, the farmers have to rely on traditional technology to increase the storage stability of milk and milk products either by converting the milk to more stable products such as butter or by treating with traditional preservatives (Lemma *et al.*, 2004).

Nowadays consumers are concerned about the synthetic chemicals used as preservatives in food, leading to a trend towards less processed food (Soomro *et al.*, 2002). In Ethiopia, there are some research reports that deal with traditional preservation methods at smallholder producer level to keep their milk and milk products quality.

### **2.2.1. Plants used for cleaning milk utensils**

Lemma *et al.* (2004) reported that about 53.3% of the women in Lume district used “Guftee” (*Sida cuneifolia*) and “Hiddii hoolotaa” (*Cucumis prophetarum*) leaves to clean milk vessels used for milking, storing and processing, while about 47 and 40% of the women in Adami Tulu and Arsi Negelle, respectively, used “Kosorata” (*Ocimum hardiense*). A lesser proportion of women used “Bargamoo adii” (*Eucalyptus globulus*). “Kosorata” (*Ocimum hardiense*), “Waahalle” (*Withania*

*somnifera*) and “Gurra harree” (*Verbascum sinaiticum*) were also commonly used as cleaning plants. Binyam (2008) also reported that 25% of the respondents in Shashemene area used Teanadam (*Ruta chalepensis*), 15% Ajubana (*Ocimum* spp.), 11% Koseret (*Ocimum hadiene*), Weira (*Olea Africana*), Tej sar (*Cymbopogon citrates*) for washing milking equipments. Leaves of shrubs or herbs such as Abalo (*Combretum molle*), Dama kese (*Ocimum suave*) and Anfar (*Buddleja polystachia*) are also reportedly used for washing milk utensils twice a day in North Western Ethiopia highland (Yitaye *et al.*, 2009).

### 2.2.2. Plants used for smoking the milk vessels

Coppock *et al.* (1992) reported that households in the semiarid pastoral system of Ethiopia smoke their milk processing vessels with burning chips of *Acacia nilotica*, *Cordia glarfa*, *Cordia ovalis* or *Combretum molle*. Alganesh (2002) also reported Dabaqaa (*Deinbollo kilimandshorica*), Baddessa (*Syzygium guineense*), Gaarrii (*Heeria reticulata*) and Ejersa (*Olea africana*) to be the most commonly used smoking plants in Eastern Wollega. According to Lemma *et al.* (2004) *Olea africana* was the most frequently used plant for smoking milk vessels in Adami Tulu, Arsi Negelle and Lume districts followed by *Juniperous procera* and *Ocimum hardiense*. These plants are used to give good flavor to the milk and milk products. Besides, they are known to increase the shelf life of milk. The use of such plants differs from place to place and even from household to household. For example, "Yuubdoo" (*Protea gaguedi*), "Hixichoo" and "Hiddii" (*Solanum spp*) were not reported from Arsi Negelle and Lume districts, while "Anshaa" (*Schefflera volkensii*), "Gatamee" (*Schefflera abyssinica*) and "Qadiidaa" were reportedly used only by small proportion of women in Arsi Negelle. Leaves of Dhirii (*Ocimum hadiense*), Beka arkte (*Lantana kamara*) (Helen, 2007) are commonly used in Kombolcha woreda, while Tena adam (*Ruta*

*graveolens*) and Adjubana (*Ocimum* species), Koseret (*Ocimum hadiense*), Weira (*Olean Africana*) and Tej sar (*Cymbopogon citrates*) (Binyam, 2008) were used in Shasemene area. According to Yitay *et al.* (2009), producers in North Western Ethiopia highlands clean milk utensils using water and leaves of shrubs or herbs such as Abalo (*Combertum molle*), Dama kese (*Ocimum suave*) and Anfar (*Buddleja polystachia*) twice a day. This is followed by drying and smoking the utensils with plants specifically mainly Qega (*Rosa abyssinica*), Keret (*Osyris quadripartite*), Tinjut (*Otostegia integrifolia*), Weira (*Olean Africana*), Tosegne (*Thymus vulgaris*) and Tid (*Juniperous procera*). According to (O'Mahony 1988; Mogessie and Fekadu, 1993) this kind of treatment plays an important role in lowering the microbial load as compared to unsmoked containers.

### **2.3. Milk processing in Ethiopia**

Milk is an ideal medium for the growth of bacteria and if it is kept at above 16<sup>0</sup>C the bacteria present will multiply rapidly thereby causing deterioration in quality and a reduction of its shelf-life (O'Connor, 1993). It is the complex mixture of water, fats, proteins, lactose, minerals, vitamins, citric acid and enzymes, together with some cells (Fox, 2003). Thus, milk processing is designed mainly to remove water from milk in order to increase its shelf life.

In Ethiopia, dairy processing is generally based on *Ergo* (Ethiopian fermented milk) where the fermentation is natural, with no defined starter cultures used to initiate it. It is thick, smooth, of uniform appearance and usually has a white milky color when prepared carefully. Raw milk is left either at ambient temperatures or kept in a warm place to ferment. The souring is brought through the proliferation of the initial milk flora; with microbial succession determined by chemical changes in the fermenting milk (Mogessie, 2002). It constitutes a primary sour milk product from which other

products may be derived. Depending on the storage temperature, it can be stored for 15 - 20 days (Almaz *et al.*, 2001) and *Lactococcus garvieae* and *Lactococcus lactis* subsp. *lactis* are the dominant species that are found in *Ergo* (Almaz *et al.*, 1999). Traditional butter (*Kibe*), traditional ghee (*Neter Kibe*), cottage cheese (*Ayib*), traditional hard cheese (*Metata Ayib*), defatted sour milk (*Arrera*), and whey (*Aguat*) are also fermented milk products manufactured in different ways in different regions (Mogess, 2006; Essayas, 2006; Zelalem *et al.*, 2007; Yitaye *et al.*, 2009). An understanding of the farmers' products and the conditions under which they operate is among the prerequisites for making improvements in the efficiency of the locally available technologies and/or adoption of new technologies by the small farmers (Fekadu, 1994).

Milk fat is the most significant solid fraction in milk. The two main products made from milk fat are butter and butter oil (Ghee). Butter yield depends on the fat content of the milk (O'Connor, 1993). Traditional butter-making in Ethiopia is an important form of extending the storage life of milk particularly during the fasting periods when dairy products are not consumed by Orthodox Christians who form the majority of the population (Tezera *et al.*, 2005). Butter can be produced by churning in bottle gourd or an earthenware jar mostly from sour whole milk. It is used as food and hair cosmetic. Out of the total produced at national level about 80% is used as food ingredient and the remaining as cosmetics (Getachew and Asfaw, 2004). According to (Zelalem *et al.*, 2011) there are three types of butter that exist in Ethiopia namely *Lega* (fresh), *Mekakelegna* (semi rancid) and *Besal* (rancid) based on the lipolysis effect due to the age of the product. All of them can be dehydrated to make ghee (*niter kibe*), which is added to a variety of Ethiopia traditional foods, in the processing different type of spices can also be added to improve the flavor.



*Arrera* (defatted sour milk) is a by- product of butter processing and has a similar color to *Ergo*, but is smoother and thinner, although thicker than fresh milk, and has a pleasant smell and taste (Zelalem *et al.*, 2011). *Arrera* can directly be consumed without further processing (Fekadu, 1994) or can be used as a raw material for *Ayib* (Ethiopian cottage cheese) production. *Ayib*-making enables good recovery of casein and residual fat and yields a marketable product. Cheese yield depends on the fat and protein contents. In addition, depending on the type of cheese being made, the ratio of fat to protein (casein) in the milk will affect the quality of the cheese (O'Connor, 1993). *Ayib* is widely consumed as side dish particularly with *Doro wet* (spicy chicken sauce) or may be spiced with herbs and spices and also mixed with (*nitir kibe*) and salt or with boiled or roasted and finely chopped cabbage and mixed with *nitir kibe*.

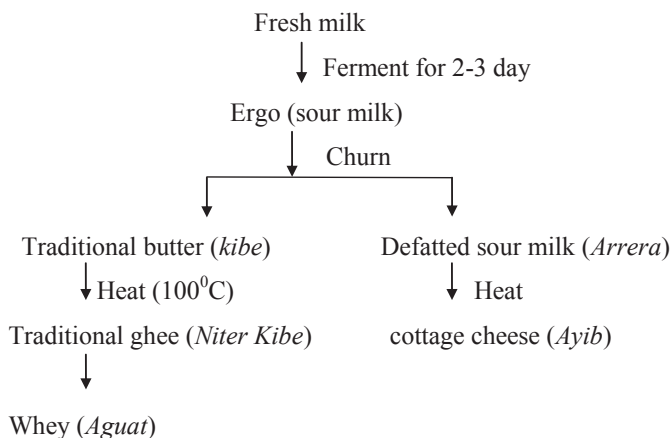
## **2.4. Cheese-making in Ethiopia**

According to (Hill, 2006), during the conversion of milk into cheese curd, milk constituents are separated into two groups; those that are retained in the curd and those that are lost in the whey. Cheese curd retains most of the fat and casein in the original milk, the discharged liquid, whey, contains most water, lactose, proteins (peptides and other nitrogenous compounds) and minerals that are soluble at the pH of cheese making. In typical bovine milk, there is approximately 3.6 g of crude protein per 100 g of milk, of which about 95% is true protein (78% caseins and 17% whey proteins) and the remainder is non-protein nitrogen (NPN) compounds. The true protein comprises the caseins, whey proteins and some casein fragments (Davies and Law, 1983). Casein is the most important protein fraction for cheese making and accounts for nearly 80% of total milk protein.

A third of the world's milk production is used to produce cheese that is of great diversity of flavor, texture and form (Fox, 2003). There are more than 1000 varieties of cheese produced throughout the world created by differences in milk source, fermentation and ripening conditions as well as pressing, size and shape. According to the British cheese Board (2003), cheese can be classified as very soft, soft, semi soft and hard.

*Ayib* is one of the Ethiopian cottage type cheese, made from sour milk after the butter is removed by churning. Milk for churning is kept in a warm place (about 30<sup>0</sup>C) for 24 to 48 hours to sour spontaneously. Churning of the sour milk is carried out by slowly shaking the contents of the pot until the butter is separated. The butter is then removed from the churn and kneaded with water. The casein and some of the unrecovered fat in defatted sour milk can be heated at a temperature between 40-70<sup>0</sup>C until a distinct curd forms. Heating the curd at 70<sup>0</sup>C for 55 minutes at pH 4 destroys most of the microorganisms (Mogessie, 1992). It is then allowed to cool gradually, and the curd is ladled out or filtered through a muslin cloth or retained in the sieve with no pressing to ensuring that there are no pockets of whey which could lead to off flavors and defects in the firmness of the curd.

According to O'Connor (1993), one kilogram of cheese can be obtained from about nine liters of butter milk or skimmed milk. Yitaye *et al.* (2009), on the other hand, reported 11 liters of defatted sour milk to be require to produce a kg of *Ayib* under smallholder settings in Northwest high land of Ethiopia.



**Figure 1.** Milk processing by smallholder dairy farmers. Source: O'Mahony and Peter (1985)

## 2.5. Quality of Cottage cheese

The term food quality includes net quantity of a unit of food, appearance (size, shape, color), flavor, aroma, texture, viscosity, shelf life stability, fitness for use as human food wholesomeness adulteration packaging and labeling (Alli, 2004). Cheese quality includes chemical composition, microbial quality and organoleptic quality (Baikhutso, 2010).

## 2.6. Chemical composition of Cottage cheese

According to Michalski *et al.* (2003), fat globule size is of primary importance for cheese quality. In soft cheese-making, the better qualitative parameters are obtained when milk contains more small-sized fat globules, whereas in producing hard cheeses, bigger milk fat globules are desirable quality attributes. A unique characteristic of cow

milk fat is the presence of short- and medium- chain fatty acids (mean about 14%) that are available sources of energy and do not deposit in human adipose tissue unlike the long chained ones (Parodi, 2004).

According to Lawrence (1993c), the casein fraction of milk protein is the dominant factor affecting curd firmness, syneresis rate, moisture retention, and ultimately affecting cheese quality and yield. As the total solids and protein content of the cheese milk increased, the resulting cheese curd had also higher protein and mineral but lower water content (Ljubica *et al.*, 2001). According to O'Connor (1993), the compositions of *Ayib* vary considerably from one smallholder farmer to another as there is no standard Ethiopian cottage cheese-making procedure. According to Mogessie (1992), *Ayib* comprises about 79% water, 15% protein, 2% fat, 1% ash, and 3% soluble milk. O'Connor (1993) also reported about 76% water, 14% protein, 7% fat and 2% ash for the same product. The pH also has great variation due to processing methods. For instance, Mogessie (1992) reported 40% of the *Ayib* samples to have pH values of less than 3.7 and 60% to have 3.7 to 4.6, while Binyam (2008), reported a pH value of 4.6 for *Ayib* samples collected from local markets and 4.34 for samples collected from producers on farm.

## **2.7. Microbial quality of cottage cheese**

The microbial quality of raw milk is crucial for the production of any high quality dairy food. Spoilage is a term used to describe the deterioration of foods' texture, color, and flavor to the point where the product is unsuitable to human consumption (Raheem, 2006). Fromm and Boor (2004) reported that microbial growth and metabolism shorten the shelf life of milk by producing undesirable changes in aroma and taste attributes that influence consumer acceptability of the products. Milk

produced under hygienic conditions from healthy animals should not contain more than  $5 \times 10^5$  bacterial/ml (O'Connor, 1994). The microorganisms that are principally involved in milk spoilage are psychrotrophic. Most psychrotrophs are destroyed by pasteurization temperatures, however, some bacteria such as *Bacillus cereus*, *Bacillus licheniformis* and *Bacillus sporothermodurans* are able to survive and grow in subsequent processing operations during cheese-making (Johnson *et al.*, 1990).

Most microorganisms that cause spoilage of cottage cheese is heat sensitive and normally do not survive the cooking process (Bigalke, 1985). Therefore, post-processing contamination by psychrotrophic bacteria can be considered to be the main reason attributing to the limited shelf life of most cottage cheeses (Weber and Broich, 1986). Soft cheese with a high pH 5-6.5 and moisture content of 50-80% may be spoiled by pseudomonas, alkali-genes and flavobacterium. The growth of these organisms in cottage cheese can cause sliminess, bitter tastes, off-flavors, and color defects, which lead to spoilage of the cheese due to proteolytic and lipolytic activities of the psychrotrophs in general and pseudomonas species in particular. According to IFST (1998), the majority of disease outbreaks related with cheese consumption are associated with the consumption of cheeses made from unpasteurized or improperly pasteurized milk. In addition, it has been recognized that post process contamination can also occur. Hicks *et al.* (1986) reported that yield losses have been significant when total count or psychrotrophic count reaches  $10^6$ cfu/ml prior to pasteurization. It should be noted that the ability of pathogens to survive and grow in cheese is detected by both intrinsic and extrinsic parameters. Intrinsic parameters of cheese include water content, pH, acidity, nutrient content, presence of antimicrobials compound and the presence of competitive microflora. These parameters vary between cheese varieties. Extrinsic parameters include factors such as processing steps, type of packaging and storage conditions (IFST, 1998).

It has been reported that the higher limit for the coliform value of cheese made from raw milk and the limit of *Enterobacteriaceae* of cheese made from heat treated milk is  $10^5$  CFU/g and  $10^3$  CFU/g, respectively under the European Union standards (CEC, 2005). Ethiopian cottage cheese (*Ayib*) is mostly made from unpasteurized sour milk. The cheese is usually made without pasteurization of milk which might lead to spoilage during storage (Abedalla *et al.*, 1993). As (Raheem, 2006) reported that soft cheeses have higher moisture content when compared to hard cheese. *Ayib* has a short shelf-life because of its high moisture content. Shelf-life can be increased by adding salt or by reducing the moisture content of the cheese, storing the product in an air-tight container also extend the storage life (O'Conner, 1993).

Soft cheeses are microbiologically unstable due to metabolic activity of bacteria, and yeast or mould contaminations (Farkye and Verdamuthu, 2002). Moulds commonly involved in cheese spoilage include members of the genera *Pencillium*, *Aspergillus*, *Cladosporium*, *Mucor*, *Fusarium*, *Monilla* and *Alernaria*. In fresh cheeses with a sufficiently high pH, such as cottage cheese, bacterial spoilage may occur. This is likely to be caused by gram-positive, psychrotrophic species, such as pseudomonades and some coliform. These organisms may contaminate the product through water used to wash the curd (Jonshon, 2002). Growth of yeasts and molds, such as *Geotrichum*, *Penicillium*, *Mucor*, and *Alternaria*, also cause spoilage of flavor, texture, and appearance (Brocklehurst and Lund, 1988). According to Robinson, (2002) *Alcaligenes viscolactis* is responsible for ropiness and sliminess in cottage cheese, while *Alcagenes metacalignes* for flat and flavorlessness. Psychotropic *Bacillus* spp. causes bitterness and proteolysis defects. According to Mogessie (1992), soft cottage cheese (*Ayib*) samples showed high numbers of mesophillic bacteria, enterococci and yeasts. The majority of the samples had mould and lactic acid bacteria counts. In

another study, mean count of 8.39, 5.36, 2.93 and 5.53 log cfu/g of aerobic mesophilic, coliform, proteolytic organisms and yeast respectively was found in *Ayib* (Zelalem *et al.*, 2005).

## **2.8. Organoleptic quality of cottage cheese**

Cattle transfer carotenoids to adipose tissue and milk, but goats, sheep and buffalos do not. Cheeses made from sheep, goat or buffalo milk are very white compared to bovine milk. It can be treated with H<sub>2</sub>O<sub>2</sub> (Fox, 2003) in order to improve the whiteness.

The properties of cheese that contribute to its texture may be divided into mechanical and geometrical. Mechanical properties are manifested by the reaction of food to stress applied during consumption (squeezing between the fingers, manual cutting, and mastication). It comprises hardness, cohesiveness, viscosity, springiness, chewiness, brittleness, and gumminess. It is measured by the pressure exerted on the cheese by the teeth, tongue, and roof of the mouth during eating. Geometrical properties include the size distribution, shape and orientation (granularity in cottage cheese). Other properties like greasiness, oiliness, succulence and mouth coating are related to the presence of fat and moisture within the cheese (Fox, 2003).

Cottage cheese is a soft, rindless and has a near white colour and granular texture consisting of discrete individual soft curd granules of relatively uniform size, from approximately 3-12 mm depending on whether small or large type of curd is desired (Codex standard 273-1968). Ethiopian cottage cheese (*Ayib*) is white in appearance and similar to European cottage cheese, but sour in taste and with a pronounced cheese flavour (Mogessie, 1990).

## **2.9. Seasoning Vegetables and Spices in Cottage Cheese (*Ayib*)**

In Ethiopia, there are a number of spices and vegetables that are added in *Ayib* in order to improve its flavour and shelf life. The spices mainly used are *Zingiber officinale* (Zingibel) and *Allium sativum* (Netch Shinkurt) (Alganesh, 2002; Binyam, 2008; Yitaye *et al.*, 2009).

### **2.9.1. Ginger**

Ginger (*Zingiber officinale*) is a rhizome with a warm, sweet, strongly aromatic odor and sharp pungent flavor. The ginger has been listed in “Generally Recognized as Safe” (GRAS) document of the USFDA. A dose of 0.5-1.0 g of ginger powder ingested 2-3 times for periods ranging from 3 months to 2.5 years did not cause any adverse effects (Polasa, 2003).

#### **2.9.1.1. Chemical composition of ginger**

Ginger (*Zingibel* in Amharic, Jinjibil in Oromifa) (*Zingiber officinale*) was originated in South East Asia. The underground rhizome of this plant, raw or processed is valued as spice (Purseglove *et al.*, 1981). The ginger rhizome contains steam volatile oil, fixed fatty acid oil, pungent compounds, resins, proteins, cellulose, pentosans, starch and mineral elements. Fresh ginger contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals, 2.4% fibre and 12.3% carbohydrate. The minerals present in ginger are iron, calcium and phosphorous. It also contains vitamins such as thiamine, riboflavin, niacin and vitamin C (Polasa, 2003). The composition varies with the type, variety, agronomic conditions, curing methods, drying and storage conditions (Govindarajan,



1982). Ebewele and Jimoh (1981) also reported that dry ginger contains 1-3% essential oil, 5-10% oleoresin, 50-55% starch and 7-12% moisture, with protein, fiber, fat and ash as its other constituents.

**Table 1.** Nutritional composition of dry ginger (per 100g)

Composition	Quantity	Composition	Quantity
Water (g)	7.0	Sodium (mg)	30
Food energy(k cal)	380	Potassium (mg)	1400
Protein (g)	8.5	Iron (mg)	11.3
Fat (g)	6.4	Thiamine (mg)	0.05
Carbohydrates (g)	72.4	Riboflavin (mg)	0.13
Ash (g)	5.7	Niacin (mg)	1.90
Calcium (g)	0.1	Vitamin activity (RE)	1.5
Phosphorus (mg)	150		

Source: (Peter, 2001)

Ginger contains a number of pungent and active ingredients. A mixture of zingerone, shogaols and gingerols volatile oils are responsible for the unique aroma and flavour of ginger and these compounds account for about 1-3% of the weight of fresh ginger (Akram *et al.*, 2011). The major pungent compounds in ginger are active gingerols which can be converted to shogaols, zingerone and paradol. The gingerols, a series of chemical homolog's differentiated by the length of their unbranched alkyl chains, were identified as the major active components in the fresh rhizome (Govindarajan, 1982), with [6]-gingerol (5-hydroxy-1-[40-hydroxy-30-methoxyphenyl] decan-3-one) being the most abundant and the compound 6-gingerol appears to be responsible for its characteristic taste and [6]-Gingerol (the main gingerol) is more pungent than [8]-

gingerol or [10]-gingerol. Zingerone and shogaols are found in small amounts in fresh ginger and in large amounts in the dried or extracted products (Gavindarajan, 1982). The shogaols, another homologous series and the dehydrated form of the gingerols, that result from the elimination of the OH group at C-5 and the consequent formation of a double bond between C-4 and C-5, are the predominant pungent constituents in dried ginger (Mustafa *et al.*, 1993).

Ginger rhizomes also contain proteolytic enzyme called zingibain (Stewart and Wood, 1991). These chemical components are influenced by factors such as the species, variety, maturity and processing conditions (Badreldin *et al.*, 2008). Huang *et al.* (2011), concluded that ginger protease possessed high milk clotting activity (MCA) and ginger protease (PA), and exhibited a higher ratio of MCA to caseinolytic activity at temperatures above 60°C, proving its potential use as a rennet substitute and accelerating agent in the dairy industry; for example, in cheese maturation and manufacture of oriental-style cheese. Su *et al.* (2009) also reported that ginger proteases could be a choice for cheese making as well as a milk-clotting enzyme source to improve on the bitterness of milk products caused by papain, ficin and bromelain and increasing the additional value of milk products.

Processing of ginger rhizomes into ginger powder resulted in decreased microbial load. The processing technique includes washing; peeling, slicing, drying in the sun and grinding into powder (Mendis *et al.*, 2009). The powdered rhizome contains 3-6% fatty oil, 60-70% carbohydrate, 3-8% crude fibre, 8% ash, 9-12% water and 2-3% volatile oil (Polasa, 2003). Oral doses of up to 2g of powdered material daily in single or divided doses are recommended over an unlimited period (Anonymous, 2003a). According to Conley (1990), fresh ginger or the ginger powder may be added to soups, stew and juices and in meat and vegetable dishes. The taste imparted to a dish

comes with the addition of ginger during cooking. It is a more subtle flavour when added at the beginning and a more pungent taste if added at the near end. Polasa (2003) also indicated that ginger is an indispensable component of curry powder sauces, ginger bread and ginger flavoured carbonated drinks. It is also used in some products like biscuits pickles and confectionaries. Furthermore, it is extensively used in preparation of dietaries for aroma and flavour. Dry ginger is used in the manufacturing of oil oleoresin, essence and processed meat. Aniedu *et al.* (2002) produced a ginger blended pineapple drink, while (Abd El-Aziz *et al.*, 2012) also fortified soft cheese with ginger extract.

#### **2.9.1.2. Antimicrobial properties of ginger**

Ginger has antimicrobial property. According to Belewu (2005), boiling or dipping West African soft cheese with ginger extract has a promising value to preserve the product. Indris *et al.* (2010) also concluded that the dipping of fish in a concentrated ginger extract before smoking has beneficial effects on the overall quality of the final products. Ficker *et al.* (2003) also indicated that ginger has antimicrobial effects against the *Staphylococci* species and also exhibits antifungal activity against a wide variety of fungi including *Candida albicans*. The report of Paramasivam *et al.* (2007) also indicated that in ginger, *Proteus mirabilis* showed maximum growth followed by *Bacillus cereus*, *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus* at 1% concentration. *Vibrio parahaemolyticus*, *Bacillus cereus* and *Proteus mirabilis* were totally inhibited at 5% concentration of ginger extract, however, *Ps. aeruginosa* showed very less growth at this concentration in fish flock. Ekeweny and Elegalam (2005) concluded that Zinger (*Zinger officinale* Rosoe) produced marked inhibitory effect on *Salmonella typhi* and *Escherichia coli* as representative of enteric microorganism. Meena (1992) also reported that fresh ginger juice showed inhibitory

action against *Aspergillus niger*, *Saccharomyces cerevisiae*, *Mycoderma* spp. and *Lactobacillus acidophilus* at 4, 10, 12 and 14%, respectively at ambient temperatures. Adesokan *et al.* (2010) indicated that adding 5% ginger powder into Ogi significantly improved its sensory attributes, leading to a relatively reduced microbial load during storage and hence an improvement in the shelf stability of the product. Kumolu-Johnson and Ndimele (2011) also concluded that ginger has some anti-oxidant and anti-fungal properties, which can retard oxidative rancidity and fungal growth, impact acceptable flavor and thus, extend the shelf life of fish like *clarian gariepinus*.

A methanolic extract of the dried powdered ginger rhizome and isolated constituents ([6]-, [8], [10]-gingerol and [6]-shogol) inhibited the growth of 19 strains of *Helicobacter pylori* in vitro (Mahady *et al.*, 2003). [10]-Gingerol and [6]-gingerdiol had potent antifungal activity against 13 human pathogens at concentrations below 1 mg/ml (Ficker *et al.*, 2003). Ginger in Sri Lanka, India and Bangladesh can also be used to treat cold, fever, stomach ache, swellings, cough and sore throat by drinking it with tea Takeda *et al.*(2007).

### **2.9.2. Garlic**

Garlic is a perennial bulb-forming plant that belongs to the genus *Allium* in the family *Liliaceae*, along with leeks (*Allium porrum*), onions (*Allium cepa*) and chives (*Allium schoenoprasum*) and used in all parts of the world as a spice and herbal medicine for the prevention and treatment of a variety of disease, ranging from infections to heart disease (Rivilin, 2001). Generally the recommended dosage for adults is 4g (one to two cloves) of raw garlic per day, one (300-mg) dried garlic powder tablet (standardized to 1.3 percent alliin or 0.6 percent allicin yield) two to three times per day, or 7.2 g of aged garlic extract per day (Ellen, 2005).

### 2.9.2.1. Chemical composition of garlic

Allicin undergoes thial- disulphide exchange reactions and can react with free thiol groups in proteins (Miron *et al.*, 2002). SH- containing enzymes so far shown to be inhibited by allicin include: succinic dehydrogenase, urease, papain, xanthine oxidase, choline oxidase hexokinase, cholinesterase, glyoxylase, triose phosphate dehydrogenase, alcohol dehydrogenase and cystine protease (Ankri *et al.*, 1997).

Allicin is sulfur containing compound (thio-2-propene-1-sulfuric acid S- allyl ester) and its production from an odorless precursor alliin, is catalyzed by an enzyme, allinase or allinlyase and responsible for the characteristic smell of garlic (Yeh and Liu, 2001). When raw garlic bulb is chopped or crushed, the enzyme allinase activates alliin, a non- protein amino acid present in the intact garlic, to produce allicin (allyl 2-proenethio sulphinate or diallyl thiosulphinate) or the enzyme allinase converts allium into the thiosulphinate allicin. According to Commission of the European Communities (2001), 1mg of allium produces 0.458 mg of allicin. On average, garlic cloves contain; 0.8% alliin. Crushed raw garlic is high in allicin, containing; 3.7 mg/g (Lawson *et al.*, 1992). Other important sulphur containing compounds present in garlic homogenates are allyl methyl thiosulphonate, 1-propenyl allyl thiosulphonate and Y-2- glutamyl-S-alkyl-L-cystine (Banjee and Maulik, 2002). Robinkov *et al.* (1994) reported that alliin is found to be the stable precursor that is converted to allicin by the action of an enzyme termed allinase, which is also present on the cloves. The transformation of allium into the biologically active allicin molecule upon crushing garlic cloves is extremely rapid, being complete in seconds. It is a volatile molecule, oily colorless liquid that is poorly miscible in aqueous solutions and comprises 70-80% of the thiosulfinates, responsible for the pungent smell of garlic and is chemically an unstable highly reactive molecule. The enzyme responsible for the lysis is allinase

or allium-lyase a pyridoxal 3-phosphatase dependent glycoprotein consisting of two subunits. Allinase is present in unusually high amounts in garlic cloves: at least 10% of the total protein content (10mg/g fresh weight). According to Pierson (1994), Allyl mercaptan is an odorous compound that is the main component of “garlic breath” after eating garlic cloves.

Although there is no standard intake of garlic, the 1988 German Commission E monograph proposed that daily intake of ~1-2 cloves garlic or ~ 4g of intact garlic may have health benefits. In many recent clinical studies, the daily dose of dehydrated garlic powder has been ~ 900mg. Yushau *et al.* (2008) said that a daily dose of 1ml/kg body weight of garlic extract for six months can result in significant reduction in oxidant (free radical) stress in the blood of patients with atherosclerosis and cholesterol circulating in the blood stream. Sasaki *et al.* (1999) reported that the dehydrated garlic in powdered or granulated form has replaced the use of fresh bulbs for industrial and home use in many countries. It is reported that about 50% of the total production of garlic is dehydrated and sent to food processors.

Garlic powder is thought to retain the same ingredients as is raw garlic; however, the proportions and amounts of various constituents differ significantly (Iberl *et al.*, 1990b). The main sulfur compound in both raw and garlic powder is alliin. On average, garlic cloves contain ~ 0.8% alliin. A pure dehydration process, with no loss of ingredients, would result in 2-2.5 mg/g alliin content in the powder. However, garlic powders contain only 1% alliin at most indicating that more than half of the alliin is lost during dehydration. Allinase is denatured by heat at a pH of < 3.5, such as that in the stomach and by many nonpolar solvents (Konch and Lawson, 1996). It is estimated that about 5kg fresh garlic bulbs from the field make 1kg of dried product

and 1g of garlic powder is equivalent to ca.2.5g of vegetable garlic with a 60% water content (O’Gara *et al.*, 2000).

**Table 2.** Nutritive composition of fresh/peeled garlic cloves and garlic powder

Nutrients	Fresh peeled garlic cloves	Garlic powder
Moisture (%)	62.8	5.2
Protein (%)	6.3	17.5
Fat (%)	0.1	0.6
Mineral matter (%)	1.0	3.2
Fiber (%)	0.8	1.9
Carbohydrates (%)	29.0	71.4
Calcium (%)	0.03	0.1
Phosphorus (%)	0.31	0.42
Iron (%)	0.001	0.004
Sodium (%)	—	0.01
Potassium (%)	—	1.1
Niacin (%)	—	0.7
Vitamin-A	0	17510/100 g
Vitamin-B (mg/100 g)	0	0.68
Vitamin-B2 (mg/100 g)	0	0.08
Vitamin-C (mg/100)	13.00	12.00
Nicotinic acid (mg/100 g)	0.4	—
Caloric value (food energy)	142 calories/100 g	380calories/100 g

Source: Pruthi (1987)

### 2.9.2.2. Antimicrobial properties of garlic

According to Bakri and Douglas (2005), garlic is a strong antibacterial agent and acts as an inhibitor on both Gram-positive and gram-negative bacteria including: *Esherichia*, *Salmonella*, *Streptococcus mutans*, *Prophyromonas gingivalis*, *staphylococuss*, *Klebisialla*, *proteus* and *Helicobacter pylori*. Raham *et al.* (2006) also reported that lactic culture was the most sensitive to the growth inhibitory active compound of garlic, followed by *Staphylococcus aureus* in contrast; *Salmonella typhimurium* and *Bacillus cereus* demonstrated the greatest resistance to garlic.

The main antimicrobial constituent of garlic has been identified as the oxygenated sulphur compound, thio-2-propene -1- sulfinic acid S-allyl ester, which is usually referred to as allicin (Curtis *et al.*, 2004). Ankri and Mirelman (1999) also revealed that the antimicrobial effect of garlic is due to the chemical reaction of the allicin with the thio-groups of several enzymes such as ARN polynase, by delaying and inhibiting microbial DNA, RNA and protein synthesis.

Different research result showed that the antimicrobial and the antifungal activity of garlic differ with the inclusion level and type of processed product of garlic. Goncagul and Ayaz (2010) concluded that garlic in fresh state, powder form and garlic oil has antibacterial, antiviral, antimycotic and antiparasitic effects. According to Adekalu *et al.* (2009), fresh *A. sativum* has antimicrobial activity against mesophilic, psychophilic and other microorganisms. According to Nickolic *et al.* (2004) garlic powder has a strong antimicrobial effect on all the microbial species tested in their study except on *Pseudomonas aeruginosa*.



Lixin and Ng (2005) isolated an antifungal protein from garlic designated alluimin, with a molecular mass of 13 KDA. Alliumin presents antifungal activity against *Mycospharella arachidicola*, inhibitory activity to the *Bacterium pseudomonas* fluorescences and exerted antiproliferative activity toward leukemia L1210 cells. The extracts of garlic also inhibit the growth of many pathogenic fungi species belonging to the genera *Aspergillus*, *Candida* and others (Carson, 1987). Yoshida *et al.* (1987) reported that ajonene compounds from garlic have stronger antifungal activity than alliicin. Similarly, ajoene compounds that are derivatives of alliicin obtained from garlic using ethyl alcohol extraction are very inhibitory against *Aspergillus Niger*, *Candida albicans* and *Paracoccidioides brasiliensis* (Naganawa *et al.*, 1996). They determined that ajoene damages the cell walls of fungi. The water extracts of garlic can inhibit in vitro *Aeromonas hydrophila* and *Streptococcus* spp. isolated from infected fish (Chatchawanchonteera *et al.*, 2008).

Ree *et al.* (1993) reported that inclusion of 1-2% garlic extract inhibits microbial growth and higher concentration is germicidal. Aqueous extracts from fresh garlic bulbs at levels of 3, 5 and 10% inhibited the growth of *Bacillus cereus* on nutrient agar plates by 31.3, 58.2 and 100%, respectively. A 5% garlic extract concentration was found to have a germicidal effect on *Staphylococcus aureus*, whereas concentrations of  $\geq 2\%$  had a clear inhibitory effect and concentrations  $< 1\%$  was not considered inhibitory (Conner, 1993). Abdl-Hafez and El-Sai (1997), on the other hand, reported garlic extract of up to 0.25% v/v have inhibitory effects on the growth of *Aspergillus flavus*, *Afumigatus*, *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus terreus*, *penicillium chrysogenum*, *penicillium puberulum*, *Pcitrinum*, *penicillium corylophilum*, *Rhizopus stolonifer*, *Stachybotrys chartarum*, *Eurotium chevalieri* and *Emericella nidulans*. According to Kung *et al.* (2002), garlic extract strongly inhibited the growth of yeasts than bacteria. Nikolic *et al.* (2004) also showed that aqueous extracts from

fresh garlic bulbs and from freshly prepared garlic powder demonstrated the greatest antimicrobial activity, while the powder aqueous extract stored at 4°C for 18 month showed a slightly weaker antimicrobial effect on all the microorganisms tested. The aqueous extract of fresh bulbs aged 4 days at room temperature almost did not contain any antimicrobial substance. However, the antimicrobial activity of the aqueous extract of garlic powder existing after a storage period of two years indicates that the extract can be applied both internally and externally for the treatment of various infections. Irikin and Korukluoglu (2007) concluded that extracts of allium vegetables can inhibit mould growth. The report of Belguith *et al.* (2010) showed that the aqueous garlic extract (57.1% (w/v) contains 324 µg/ml allicin.

The garlic extract inhibits the growth of some bacterial strains like *Escherichia coli*, *Salmonella aureus* and *Salmonella* spp. This action may be due probably to the antimicrobial properties of garlic extract that are caused by the presence of active ingredients (allicin and cinnamaldehyde) present in garlic extract (Belewu, 2005). From the result of Leuchner and Zamparini (2002) study, 1% w/v of garlic showed bacteriostatic and *Salmonella enterica* and *Escherichia coli* O157, and *Escherichia coli* were more sensitive. Paramasivum *et al.* (2007) reported that in 1% garlic and ginger extract, there was a bacterial growth; however, with 5% concentration bacterial growth was suppressed probably by inhibiting the coagulase activity of the bacteria. Lorowitz and Clark (1982) revealed that the storage shelf life of ground camel meat treated with 5% or more of ground garlic was extended. Belguith *et al.* (2010) also suggested that garlic, as a natural herb, could be used to extend the shelf life of meat products, providing the consumer with food containing natural additives, which might be seen more healthful than those of synthetic origin. According to the report of Adekalu *et al.* (2009), a tomato puree treated with 4-5% *Allium sativum* was stored for 6-10 days, while its shelf life decreased at 3% and when treated with 1-2% *Allium*

*sativum*, the product was stored for only two days. As indicated by Deresse (2010), diluted solution of garlic can completely inhibit the growth of *Staphylococcus aureus* at a concentration of more than 7.50mg/ml. However, the same authors reported the effect of different levels of pH on garlic activity to be not apparent. Garlic contains powerful antioxidants that can extend the shelf life of dry fermented sausages (Nassu *et al.*, 2003).

Naturally, heating at high temperatures decreases the antimicrobial activity of garlic due to the inactivation of the enzyme alliinase by heat (Lawson, 1996). Dried powdered garlic contains approximately 1% alliin, which is broken down to allicin when the garlic bulb is crushed or cut. Further conversion yields ajoene. Aged garlic (to reduce the odour) or steam distilled garlic (to produce garlic oil) denatures allicin and thus decreases activity. Aged garlic and garlic oil do not contain significant amounts of alliin or allicin and as a result do not have much physiologic activity as fresh garlic or garlic powder. Generally, fresh garlic produced the greatest inhibition followed by freeze dried powder and the antimicrobial activity of dried powder decreased with decreasing concentration. Garlic powder has a different taste from fresh garlic. If it used as a substitute for fresh garlic, 1/8 teaspoon of garlic powder is equivalent to one clove of garlic. According to Whitemore and Naidue (2000), in addition to its nutritional effects, the antibacterial and antifungal activities of garlic against a variety of Gram-negative and gram-positive bacteria were and are still important, and continue to be extensively investigated.

## **2.10. Sensory properties of cheese**

Texture, color, taste, aroma and visual appearance can be used to define the sensory food quality (Di Monaco *et al.*, 2008). The aroma/flavor and textural characteristics of

cheese depend heavily on the method of production, type of milk and metabolic activities of selected starter organisms, and also diversity of species and strains of local and specific indigenous milk microflora (Mijacevic and Bulajic, 2008).

Cottage chesses possess a pleasant and desirable flavor similar to fresh whole milk or cream and also delicate flavor and aroma of lactic acid and diacetyl. Flavoring ingredients shall be uniformly distributed throughout the product. And also have a meaty texture, but if creamed sufficiently tender to permit proper absorption of cream or creaming mixture. The texture should be smooth, velvety, not meaty, crumbly, pasty, sticky, mushy, and watery or slimy. The product should not possess any other objectionable characteristics of body and texture, but, should present a clean, natural and creamy white color (USDA, 2001).

The moisture, fat, salt and mineral particularly calcium contents, pH and proteolysis activity dictate the resultant functionality of a given cheese variety. These properties play heavily on consumer perceptions of cheese quality (McMahon and Oherg, 1998). Texture, color, taste, aroma and visual appearance can be used to define the sensory food quality (Di Monaco *et al.*, 2008). Each cheese variety has its specific sensory attributes, which are widely appreciated by consumers (Barcnas *et al.*, 2004). The traditional character of a cheese and its designation of origin are two of the most important factors influencing consumers' preference in the market (Bertozzi and Panari, 1993).

Primary degradation of milk constituents leads to the formation of a whole range of precursors of flavor compounds. Some compounds formed by glycolysis (lactose and citrate), lipolysis (milk lipids) and proteolysis (caseins), directly contribute to cheese flavor (short- chain fatty acids, acetaldehyde diacetyl, peptide and aminoacids.

Primary degradation of major caseins,  $\alpha$ s1- caseins, has major consequences for cheese texture and also compounds arising from the catabolism of free amino acids that contribute directly to cheese taste and aroma (Singh *et al.*, 2003).

The role of pH in cheese texture is important due to changes in the protein network of the cheese curd. As the pH of the cheese curd decreases, there is a concomitant loss of colloidal calcium phosphate from the casein micelles and below about pH 5.5, a progressive dissociation of the sub-micelles into smaller aggregates occur (Lawrence *et al.*, 1987). Curds for different cheese varieties are recognizably different at the end of manufacture; mainly as a result of compositional and textural differences in milk composition and processing factors. The pH at whey drainage largely determines the mineral content of cheese. The loss of  $\text{Ca}^{2+}$  and phosphate from casein micelles determines the extent to which they are disrupted, and this largely determines the basic structure and texture of a cheese (Lawrence *et al.*, 1983).

The flavor of cheese occurs due to the presence of chemical components, such as diacetyl and acetate, which originate from the degradation of milk citrate by mesophilic starter culture (Baikhutso, 2010). The flavor is a component of taste and aroma. Taste refers to the water soluble fractions, which include peptides, amino acids, organic acids, salts and amines. The volatile fraction of cheese has several sulfur-containing compounds such as methanethiol, methional, dimethyl sulfide, dimethyldisulfide, dimethyltrisulfide, dimethyltetrasulfide, carbonyl sulfide and hydrogen sulfide (Urbach, 1995) and they contribute to the aroma of cheese (Milo and Reineccius, 1997). Cheese aroma can be natural, which is originating from enzymatic activity of the starter microbes or acquired, which can due to the addition of other ingredients in the finished products (Scott, 1998).

A high fat content in milk inhibits the process of syneresis during cheese making, resulting in higher moisture cheese (Lawrence *et al.*, 1984). Chen *et al.* (1979) concluded that fat content had little influence on the hardness of a wide variety of natural and processed cheeses.

### **2.11. Summary of the literature review**

Based on the literature review it can be observed that milk and milk products play a significant role in the nutrition as well as income generated at national and household level. However, though a substantial value can be added to milk using available resources, very limited work has been conducted thus far thus the benefits being utilized from this valuable product is much below potential. A number of earlier studies at different corners of the globe indicated that the sensory as well as microbial qualities of various types of cheese can be improved by using different parts and extracts (in different forms) of a number of locally available plant species. Such innovative practices not only diversify products but also improve their consumer acceptance thus increase the income generated from such products by the engaged. The current study aims at contributing to filling the existing knowledge gap in making use of locally available resources to improve the sensory as well as microbial and shelf life of *Ayib* (Ethiopian cottage cheese).

### **3. MATERIALS AND METHODS**

#### **3.1. Description of the Study area**

The study was conducted at the Dairy Technology Laboratory of Holetta Agricultural Research Center of the Ethiopian Institute of Agricultural Research (EIAR). The center is located at 9°3' N latitude and 38° 30' E longitudes, at about 35 Km West of Addis Ababa along the main road to Ambo. The study area is situated at an altitude of 2400 m.a.s.l. and receives mean annual rainfall of 1100 mm. The mean minimum and maximum temperatures of the area are 6°C and 24°C, respectively (Zelalem *et al.*, 2005).

#### **3.2. Source and Preparation of Garlic and Ginger Samples**

Matured ginger (*Zingiber officinale*) Yali variety and fresh head of garlic (*Allium sativum*) Bishoftu netch variety was purchased from Jimma and Debre Zeit Research Center, respectively.

##### **3.2.1. Ginger powder preparation**

Ginger powder was prepared following the procedure described by Sukaing *et al.* (2010). Briefly, fresh mature rhizomes of ginger were sorted, thoroughly washed, peeled and sliced (about 2 mm thickness) with a sharp knife. These sliced rhizomes were then sun dried with final moisture reached 10%. Then after, the dried gingers were ground by using electric kitchen grinder. Finally, the powder was screened through sieve.

### **3.2.2. Garlic powder preparation**

Garlic powder was prepared according to the method described by Douglas *et al.* (2005). The outer cover of garlic cloves were peeled off; washed with clean water; and sliced with sharp knife. The sliced cloves were sun dried with final moisture 10%. The dried slices were ground by electric kitchen grinder. The garlic powder was then screened through sieve as it was done with ginger. Both ginger and garlic powders were packed in sterile glass bottles and stored in dark and clean area (Douglas *et al.*, 2005).

### **3.3. Ayib – making**

Fresh whole cows' milk was collected from Holeta Agricultural Research Center dairy farm in nine batches of 270 liters. Milk quality was examined at each sampling using (clot-on-boiling) by using 5ml of milk in the test tube and placed in boiling water for minutes then examined for precipitate and organoleptic test (flavor and color). *Ayib* was prepared according to the procedure described by Mogessie (1990b) and Zelalem *et al.* (2007). Raw milk was kept at room temperature for 48 h to sour spontaneously. When adequate souring was achieved and the pH of soured milk lowered to 4.0, it was churned by electric churner (SN 0794, Italy) to recover the butter. Then after, the defatted sour milk obtained as a byproduct of churning (butter-making) was heated to a temperature of 50°C for 55min and cooled overnight. The whey and curd were separated by using a sieve and the curd was retained in the sieve for about an hour for effective drainage. The *Ayib* was then collected and weighed. Each *Ayib* sample was packed in sterile bottles, sealed with aluminum foil and stored for different tests.



**3.4. Experimental Layout**

The experimental layout was a complete randomized design (CRD) with three replications. Ginger, garlic and their 1:1 ratio mixture powder with (1, 3 and 5%) was added into the *Ayib* after curd cooking and draining the whey (Conely, 1997). A control (*Ayib* without spices) was also considered. The weight and volume both *Ayib* and whey were measured. Each treated *Ayib* and the control (*Ayib* without spices) were kept at ambient temperature for the experiment. All *Ayib* samples were examined for microbial, pH and chemical composition every 24h starting from 0 hour (right after processing) for 10 days.

**Table 3.** Experimental layout

Treatment	Levels	Replication
Garlic	1	3
	3	3
	5	3
Ginger	1	3
	3	3
	5	3
Garlic/Ginger	0.5/0.5	3
	1.5/1.5	3
	2.5/2.5	3
Control	0	3

### 3.5. Determination of chemical composition of *Ayib*

#### 3.5.1. Total solid

The total solid of *Ayib* was determined by oven drying method (AOAC 948.12, 1995). Duplicate 5 gm *Ayib* samples were weighed in pre dried and weighed crucible dishes and dried in oven drier (Dv 10621, England) at 110<sup>0</sup>C for 24 h. They were then cooled and reweighed. The total solids content was calculated using the following formula:

$$Total\ solids\ (TS\%) = \left( \frac{weight\ of\ dried\ sample}{sample\ weight} \right) \times 100$$

#### 3.5.2. Ash determination

The ash contents of *Ayib* samples were determined according to the method described in AOAC (1995) method No. 935.42. Using the dried *Ayib* samples from the determination of total solids content, a sample placed into a Muffle Furnace, and ignited at  $\leq 550^0$ C until the ash was carbon free. Then, it was placed in a desiccator for cooling and re-weighed. The initial and final weights of the sample were taken. The obtained ash weight was divided by original sample weight and expressed in percent. The ash content was calculated using the following formula:

$$Ash\ \% = \frac{weight\ of\ residue}{weight\ of\ sample} \times 100$$

### 3.5.3. Fat determination

#### *Gerber method*

The fat content of *Ayib* was determined using the procedure described by Richardson (1985). A 3 g of *Ayib* sample was weighed on dried and tared duplicate aluminum foil. 10 ml of sulfuric acid was pipetted into duplicate butyrometers without wetting the neck of the tube. Then, 3 ml of distilled water was first added in each butyrometer then an additional 4 ml of water was added to homogenize the sample portion. One ml of amyl alcohol was then added into the butyrometer, which was then tightly closed with a lock stopper, and shaken by inverting several times until all the *Ayib* sample portion was digested by the acid. Then after, the butyrometer was kept in a water bath for 5 min at 65°C and centrifuged in a Gerber Centrifuge for 5 min. The butyrometer was placed again in water bath at 65°C for 5 min and removed to make and record the butyrometer reading.

### 3.5.4. Determination of acidity of *Ayib*

The acidity of *Ayib* samples was determined according to the method described in AOAC (1995) method No. 920.124. A 100 ml of distilled water warmed at 40°C was added into 10g *Ayib* sample in duplicate flasks (105ml); then it was stirred vigorously and filtered using filter paper (Whatman No.1). A 25ml portion of the filter that represented 2.5g of the *Ayib* sample was titrated with 0.1N NaOH using phenolphthalein as indicator.

The result was expressed as % lactic acid and calculated using the following formula

$$\% \text{ lactic acid} = \frac{\text{ml of 0.1 N NaOH used} \times 0.09}{\text{ml of filtrate of Ayib sample used}} \times 100$$

### **3.5.5. pH determination**

The pH of the *Ayib* samples was measured using a digital pH meter after calibrating using standard buffer solutions of pH 4 and 7. The pH was measured by inserting the pH and temperature probe together into the sample and reading was taken when the displayed value was steady.

### **3.6. Microbiological Analysis**

The microbiological quality tests for the *Ayib* samples considered include: Aerobic mesophilic bacteria count (AMBC), coliform count (CC) and yeast and mould count (YMC). Standard Plate Count Agar (SPCA) (Oxoid) was used to determine Aerobic mesophylic bacteria count; while Violet Red Bile Agar (VRBA) (Oxoid) was used for Coliform count; and Potato Dextrose Agar (PDA) (Oxoid) for Yeast and Mould. Each of the tests was made in three replicates and a control was prepared for each analysis. For AMBC, dilutions were selected so that the total number of colonies on a plate was between 30- 300, while for CC dilutions were selected for plate counts of between 15- 150 (Richardson, 1985).

Peptone water and the culture media used for each count were autoclaved for 15 min at 121<sup>0</sup>C, except Violet Red Bile Agar (VRBA), which was boiled for about 2 min until it was completely dissolved (Richardson, 1985). Media used were prepared according to the directions given by the manufacturers.

### **3.6.1. Aerobic mesophilic bacteria Count (AMBC)**

AMBC was determined by the method described by Richardson (1985). Briefly, 1g of the *Ayib* sample was diluted in 9 ml of peptone water to obtain an initial  $10^{-1}$  dilution level and 1 ml of the initial dilution was pipetted into a test tube that contains 9 ml of 0.1% peptone water. 10 ml of the media was added into a sterile Petri dish and allowed to solidify. Zero point one ml of at  $10^{-5}$  and  $10^{-6}$  dilution levels of the *Ayib* samples were surface plated over the solidified medium. The plates were then incubated at  $32^{\circ}\text{C}$  in inverted position and AMBC was done after 48 hours of incubation.

### **3.6.2. Coliform Count (CC)**

CC was determined according to the method described by Richardson (1985). Zero point one ml at  $10^{-2}$  and  $10^{-3}$  dilution level was surface plated and incubated and CC was done after 24h of incubation. Typical dark red colonies ( $>0.5\text{mm}$  in diameter) were considered as coliforms. This was confirmed by transferring five typical colonies from each plate to tubes of 2% Brilliant Green Lactose Bile Broth (BGLBB) (Oxoid). Gas production after 24 hours of incubation at  $32^{\circ}\text{C}$  was considered as sufficient evidence for the presence of coliforms.

### **3.6.3. Yeast and Mould Count (YMC)**

Yeast and mould count was determined as per the procedure described by Marth (1978). Decimal dilutions of *Ayib* samples at  $10^{-6}$  and  $10^{-7}$  were made in the same manner for AMBC and CC described above. YMC were made after incubating plates at  $25^{\circ}\text{C}$  for 5 days. Colony count was calculated by using the formula (IDF, 1991).

$$count = \frac{\Sigma C}{v(n1 + 0.1n2)} * d$$

Where: N = Number of cfu/g or mL of test sample

ΣC= sum of all colonies counted (between 10-300)

n1 = number of plates from the lowest dilution used for computing the count

n2= number of plates in the next dilution factor used for computing the count

d= reciprocal of the dilution factor of used for computing the count corresponding to n1

v= is the volume of sample applied in each plate

### **3.6.4. Shelf life determination**

The controls and treated samples of *Ayib* were stored at ambient temperature for 10 days; and 1 g of the control and treated *Ayib* samples were taken every 24 hours for microbial examination (Collin and Mustafa, 1969). Organoleptic evaluation was done by 5 panelists as per recommended by Hassen *et al.* (1983) every 24 hours, and the product is considered to be spoiled when the product is discarded by at least 50% of the evaluators, that date discretion is considered as a failure date (Schmidt and Bouma, 1992). The shelf life of the samples was taken to be one day less the failure date.

### **3.7. Sensory evaluation**

Sensory evaluation of *Ayib* samples by consumer panelists was conducted according to the method described by Resurrecin (1998). Testing was conducted at Dairy Technology Laboratory of the Holeta Agricultural Research Center. Fifty-eight adult consumers (23 men and 35 women) were asked to fill the questionnaire prepared to evaluate the sensory attributes of the *Ayib* samples. Consumer panelists were selected

based on the following criteria: age between 18-64 years old and they had to be “consumers” of fermented milk products. *Ayib* samples (20g) were placed in a three digit coded white plastic plates and served in a bright well ventilated room. Distilled water was provided to the panelists to rinse their mouth after each taste. The sensory attributes of *Ayib* samples i.e taste, color, aroma, texture, appearance and overall acceptability were evaluated using a 5-point Hedonic scale (5=like very much, 4= like moderately, 3=neither like nor dislike, 2=dislike moderately and 1=dislike very much). Each *Ayib* samples were presented in a randomly fashion.

### 3.8. Data analysis

The data generated from the physico-chemical and microbial analyses were analyzed using the General Linear Model procedure of the Statistical Analysis System software (SAS, Version 9). The data on microbial counts were first transformed to logarithmic ( $\log_{10}$ ) values before subjected to statistical analysis. Sensory score data were analyzed by using the analysis of variance technique.  $P < 0.05$  was considered as the level of significance using Duncan’s multiple ranges.

Failure data were analyzed by using Weibull distribution method to model the shelf-life data as used by Schmidt and Bouma (1992). Nominal shelf-life was calculated from this distribution fit characteristics for each treatment.

The Weibull probability distribution function is given by:

$$f(y) = \frac{\omega}{\varphi} \left(\frac{y}{\varphi}\right)^{\omega-1} \exp\left\{-\left(\frac{y}{\varphi}\right)^{\omega}\right\}$$

where  $\omega > 0$  is shape parameter,  $\varphi > 0$  is scale parameter,  $y \geq 0$ . The cumulative distribution is:

$$F(y) = 1 - \exp\left\{-\left(\frac{y}{\varphi}\right)^{\omega}\right\}$$



## 4. RESULTS AND DISCUSSION

### 4.1. Physicochemical Properties of *Ayib*

The result of proximate composition, pH and titratable acidity of the *Ayib* samples are presented and discussed in the subsequent sections.

#### 4.1.1. Proximate composition of *Ayib*

Treating *Ayib* samples with different levels of ginger, garlic and their mixture powder had no significant ( $p>0.05$ ) effect on the % contents of total solid, ash and fat of the samples (Table 4). This current result is in line with reports of a number of earlier related studies. Sallam *et al.* (2004), for instance, revealed that the addition of garlic in different forms (fresh, powder and oil) did not show marked effect on protein, moisture and fat contents of chicken sausage. Rabita *et al.* (2006) also showed the non apparent effect of cardamom powder, thyme powder and clove powder addition on moisture, fat, salt and total nitrogen contents throughout the 45 days storage time of white soft cheese made from heated goat's milk. Kumolu-Johnson and Ndimele (2011), however, reported a different result where a marked difference in ash content was observed when fresh ginger was included in various levels in cat fish. Gundogdu *et al.* (2009) also reported a significant difference in dry matter content of stirred type of yoghurt when garlic was included in different levels. The contents in % of total solid and ash of control *Ayib* samples were observed to be the lowest values of all treated *Ayib* samples. This might be due to the difference in the treatment ratio. A number of earlier works also reported lower mean values of % total solid, ash and fat contents for *Ayib* samples as compared with that of the current study (Mogessie, 1992;

Fekadu, 1994; Zelalem *et al.*, 2007; Binyam, 2008). This might be due to the difference in composition of the raw material and the spices.

No significant difference ( $p>0.05$ ) was observed on total solid, ash and fat contents of the Ayib samples throughout the storage period among the four treatments (ginger, garlic and their mixture powder treated and control). This result is in line with Sallam *et al.* (2004), who reported no significant effect of different garlic powder inclusion on the moisture and fat contents of chicken sausage over the storage days. However, Alalade and Adeneye (2006) reported decreasing fat content of Wara (Nigerian soft cheese) as the storage period advances. Gundogdu *et al.* (2009) also indicated decreasing fat content of garlic treated yoghurt at 1% than 0.5% level of inclusion and as storage time advances. Dewi *et al.* (2010) also reported a significantly ( $p<0.05$ ) higher moisture, protein and fat contents of duck sausage formulated with 1.5% garlic powder after 21 days of storage time compared with the original contents.

**Table 4.** Effect of ginger, garlic and their mixture inclusion (%w/w) on proximate composition of *Ayib* samples (Mean  $\pm$  SE)

<b>Treatment</b>	<b>Fat</b>	<b>Ash</b>	<b>Total solid</b>
Control	1.35 $\pm$ 0.18	1.24 $\pm$ 0.09	25.50 $\pm$ 1.64
1%Ginger	1.43 $\pm$ 0.28	1.34 $\pm$ 0.13	27.2 $\pm$ 2.33
3%Ginger	1.52 $\pm$ 0.16	1.34 $\pm$ 0.07	27.27 $\pm$ 1.29
5%Ginger	1.32 $\pm$ 0.26	1.35 $\pm$ 0.04	27.29 $\pm$ 1.13
1%Garlic	1.50 $\pm$ 0.22	1.39 $\pm$ 0.15	27.02 $\pm$ 2.22
3% Garlic	1.31 $\pm$ 0.13	1.35 $\pm$ 0.08	27.78 $\pm$ 1.36
5% Garlic	1.18 $\pm$ 0.14	1.36 $\pm$ 0.06	27.67 $\pm$ 1.31
1%Mixture	1.45 $\pm$ 0.25	1.29 $\pm$ 0.11	27.62 $\pm$ 2.51
3% Mixture	1.29 $\pm$ 0.11	1.34 $\pm$ 0.09	27.41 $\pm$ 1.91
5% Mixture	1.16 $\pm$ 0.16	1.39 $\pm$ 0.05	27.60 $\pm$ 1.13

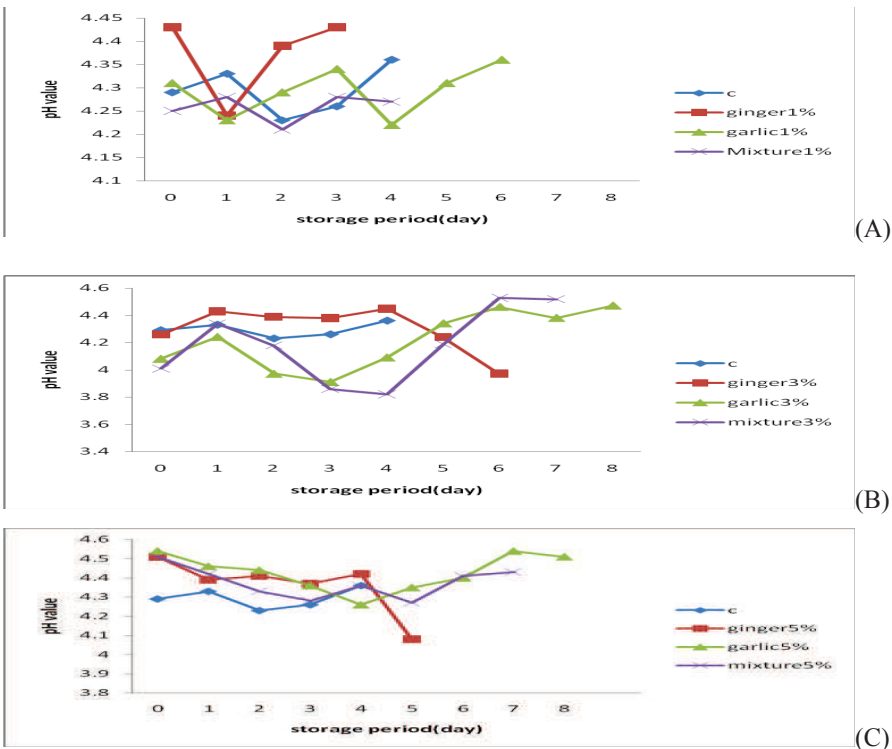
#### 4.1.2. pH and Titratable Acidity

Treating *Ayib* samples with ginger, garlic and their 1:1 mixture powder did not show significant ( $p>0.05$ ) effect on the pH value of the samples (Table 5). Though not marked, the pH of *Ayib* samples ranged from the lowest (4.18) for 1% garlic-ginger mixture treated *Ayib* samples to the highest (4.37) for 5% mixture treated *Ayib* samples. Similarly, ginger, garlic and their mixture (1:1 ratio) treatment did not show significant effect on titratable acidity of *Ayib* samples (Table 5). This might be due to the antimicrobial activity of the spices. This result is in line with that of Foda *et al.* (2010) who revealed that higher spearmint oil concentration did not affect the titratable acidity of white cheese. *Ayib* samples with the highest spice % inclusion showed high pH value leading to slow acid development in the samples. This result agrees with that of Rabita *et al.* (2006) who indicated white cheese with 0.2% cardamom to have shown the slowest acid development compared with that with 0.1 and 0.15% thyme powder, 0.1 and 0.2% clove powder and 0.1% cardamom powder.

**Table 5.** Effect of ginger, garlic and mixture powder treatment on pH and titratable acidity of *Ayib* (Mean $\pm$  SE)

Treatment	pH	Titratable acidity
Control	4.29 $\pm$ 0.04	0.68 $\pm$ 0.08
1%Ginger	4.36 $\pm$ 0.03	0.76 $\pm$ 0.12
3%Ginger	4.30 $\pm$ 0.05	0.79 $\pm$ 0.12
5%Ginger	4.33 $\pm$ 0.04	0.79 $\pm$ 0.17
1%Garlic	4.28 $\pm$ 0.05	0.71 $\pm$ 0.11
3% Garlic	4.28 $\pm$ 0.11	0.77 $\pm$ 0.09
5% Garlic	4.30 $\pm$ 0.04	0.78 $\pm$ 0.16
1%Mixture	4.18 $\pm$ 0.09	0.72 $\pm$ 0.11
3% Mixture	4.23 $\pm$ 0.09	0.73 $\pm$ 0.08
5% Mixture	4.37 $\pm$ 0.04	0.73 $\pm$ 0.14

*Ayib* treated with 1% ginger, 1% garlic and 5% ginger powder showed significantly ( $p < 0.001$ ) decreasing pH value from 0 to 1<sup>st</sup> days of storage, while 5% garlic and 5% mixture powder showed decreasing trend from 0 to 4 and 0 to 3 days of storage period, respectively (Figure 2). The current result agrees with the report of Gundogdu *et al.* (2009) where yoghurt samples treated with 1% garlic showed higher pH value than that with 0.5% during their storage periods. This might be due to the effect of the spices that may have affected the lactic acid activity (Raham *et al.*, 2006). Then after, the pH of the *Ayib* samples tended to gradually increase in all cases except (3 and 5%) ginger powder treated *Ayib* sample that showed declining trend. Such decreasing trend of pH during the storage period might be accounted to the action of psychrotrophic bacteria that ferment the carbohydrate present in the binders and spices. Metry *et al.* (2007) also showed that the pH of white soft cheese samples treated with cardamom, thyme and clove essential oil significantly ( $p < 0.05$ ) decreased during the pickling period (45 days) in all samples. Moreover, Gundogdu *et al.* (2009) reported that the pH value of garlic treated stirred and set type of yoghurt significantly declined during the storage period. Adesokan *et al.* (2010) also revealed that the pH value of Ogi (Nigerian fermented food) treated with ginger powder at (1, 5 and 10%) significantly ( $p < 0.05$ ) decreased throughout the fermentation period. In the present study, the titratable acidity of the *Ayib* samples did not show significant change throughout the storage periods, which deviated from the values reported by (Osman and Omer, 2008) who indicated a significant ( $p < 0.05$ ) increase during 240 days storage time of Sudanese white cheese. A marked ( $p < 0.05$ ) increase in titratable acidity of white soft cheese samples treated with cardamom, thyme and clove essential oil during the pickling period (45 days) was also reported (Metry *et al.*, 2007).



**Figure 2.** Effect of ginger, garlic and mixture (1:1 ratio of ginger and garlic) powder on pH value of Ayib during the storage period. (A) 1% inclusion, (B) 3% inclusion and (C) 5% inclusion, C = Control.

#### 4.2. Microbial properties of Ayib

Spice powder treatment did not significantly ( $p > 0.05$ ) affect AMBC, YMC and CC of the *Ayib* samples (Table 6). Though not significant ( $P > 0.05$ ), the lowest YMC was observed for *Ayib* samples treated with 3% garlic and also the lowest CC was observed for samples treated with 3% garlic powder with the highest values for the

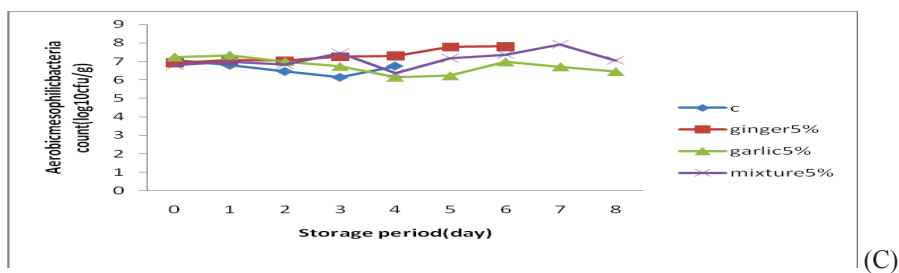
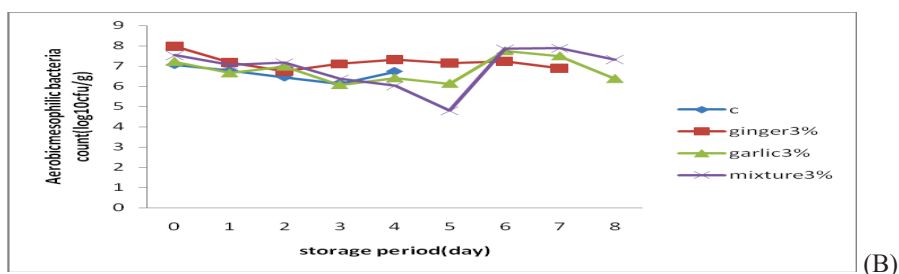
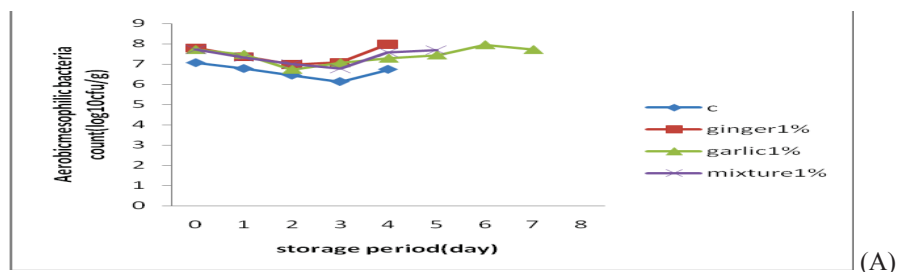
three counts (AMBC, YMC and CC) being observed in samples from the 1% garlic, 3% ginger powder and 5% ginger powder treated group respectively. This might be due to the low antimicrobial effect of ginger and also 1% garlic powder might not be sufficient to prevent the microbial growth that are found in *Ayib* samples. Among treatments, the lowest YMC and CC were observed for *Ayib* samples treated with 3% garlic powder. As indicated by Paramasivam *et al.* (2007), this might be due to the high antimicrobial effect of garlic compared with the other treatments considered. Sallam *et al.* (2004) also reported the addition of fresh and powdered garlic to have decreased Aerobic plate count of chicken sausage.

**Table 6.** Microbial counts (Mean  $\pm$  SE in log<sub>10</sub> cfu/g) in *Ayib* samples treated with different levels of ginger, garlic and their mixture

<b>Treatment</b>	<b>Aerobicmesophilic bacteria count</b>	<b>Yeast and mould count</b>	<b>Coli form count</b>
Control	6.62 $\pm$ 0.52	7.43 $\pm$ 0.58	1.96 $\pm$ 0.37
1%ginger	7.33 $\pm$ 0.26	7.35 $\pm$ 0.79	1.62 $\pm$ 0.36
3%ginger	7.07 $\pm$ 0.32	8.25 $\pm$ 0.31	2.23 $\pm$ 0.78
5%ginger	7.08 $\pm$ 0.47	7.82 $\pm$ 0.35	2.83 $\pm$ 0.53
1%garlic	7.42 $\pm$ 0.09	8.01 $\pm$ 0.39	2.14 $\pm$ 0.15
3% garlic	6.79 $\pm$ 0.25	6.32 $\pm$ 0.45	1.09 $\pm$ 0.47
5% garlic	6.75 $\pm$ 0.25	7.32 $\pm$ 0.59	1.71 $\pm$ 0.16
1%mixture	7.29 $\pm$ 0.34	7.82 $\pm$ 0.81	2.39 $\pm$ 0.58
3%mixture	6.75 $\pm$ 0.51	7.85 $\pm$ 0.35	2.19 $\pm$ 0.59
5%mixture	7.00 $\pm$ 0.45	7.20 $\pm$ 0.75	2.59 $\pm$ 0.21

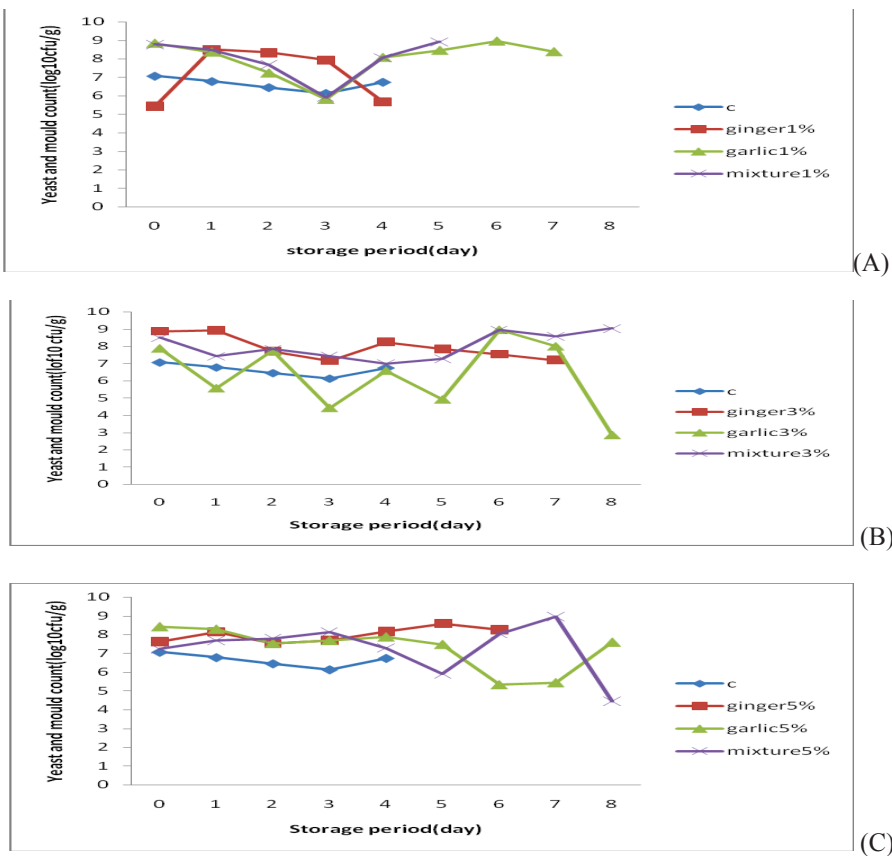
Spice powder treatment at different percent inclusion also did not show significant ( $p>0.05$ ) effect on AMBC, CC and YMC of the *Ayib* samples over the storage period. This can be explained by the similar storage temperature that was chosen based on the prevailing storage practice of the local community. This present result agrees with that

of Kumolu-Johnson and Nidimele (2011) who indicated the absence of a marked difference in microbial loads in fish with different levels of garlic treatment over a storage period of 28 days. Though not significant, all the coliform counts tended to decrease gradually *Ayib* samples treated with 5% garlic powder being more pronounced (Fig 5C). This decrease could be attributed to the antimicrobial effects of the garlic powder that suppress the growth of microorganisms coupled with the gradual increase in pH value of the *Ayib* samples. This result is also in line with that of Rabita *et al.* (2006) who indicated that CC of white cheese treated with cardamom, thyme and clove powder decreased from day 30 of the storage period through day 45. Metry *et al.* (2007) also reported that CC of white cheese treated with cardamom, thyme and clove essential oil decreased throughout the storage period.

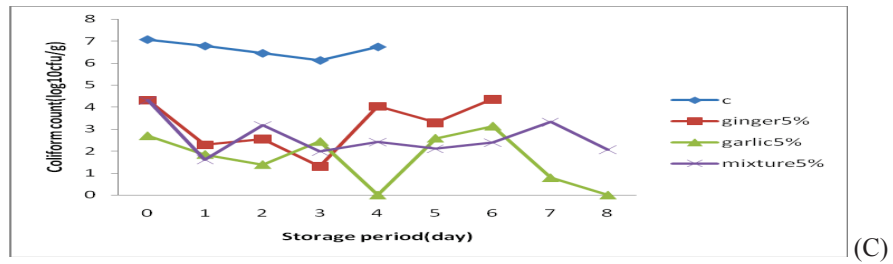
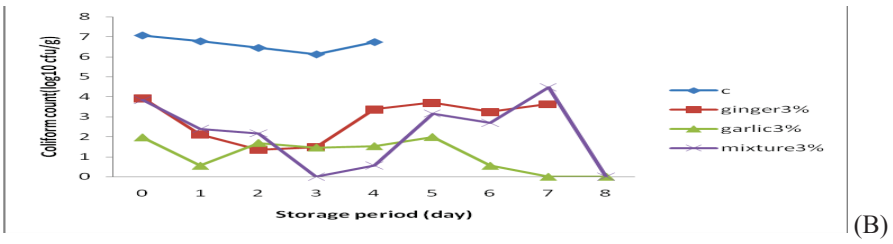
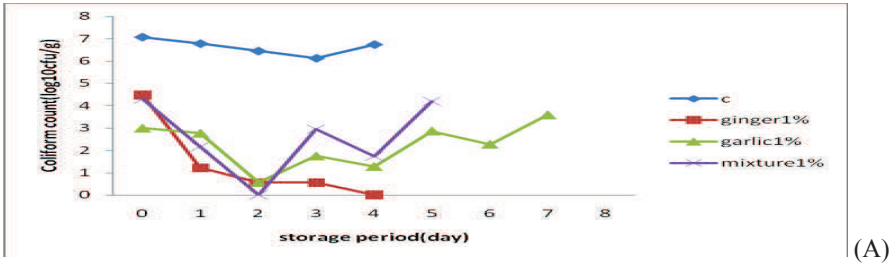


**Figure 3.** Effect of ginger, garlic and mixture (1:1 ratio of ginger and garlic) powder on the aerobic mesophilic count of *Ayib* during the storage period. (A) 1% inclusion, (B) 3% inclusion and (C) 5% inclusion. C= control.





**Figure 4.** Effect of ginger, garlic and mixture (1:1 ratio of ginger and garlic) powder on yeast and mould count of *Ayib* during the storage period. (A) 1% inclusion, (B) 3% inclusion and (C) 5% inclusion. C= Control.



**Figure 5.** Effect of ginger, garlic and mixture (1:1 ratio of ginger and garlic) powder on coli form count of *Ayib* during the storage period with different % inclusion. (A) 1% inclusion, (B) 3% inclusion, (C) 5% inclusion. C= Control

### 4.3. Organoleptic Properties

The mean value of taste, aroma, color, appearance and texture of the *Ayib* samples are indicated in Table 7. Spice powder treatment significantly ( $p<0.05$ ) affected the color, appearance and texture of the *Ayib* samples as judged by panelist. However, no significant ( $P>0.05$ ) difference was observed in taste and aroma. This might be attributed to the respective taste and flavor of ginger and garlic powders that might have equally influenced scores given by the panelists on the taste and flavor of the *Ayib*.

**Table 7.** Organoleptic property of *Ayib* samples treated with ginger, garlic and their mixture

Variable	Taste	Aroma	Color	Appearance	Texture
Control	3.95	4.09	4.61 <sup>a</sup>	4.35 <sup>ab</sup>	4.23 <sup>ab</sup>
1%ginger	3.61	4.41	4.36 <sup>ab</sup>	4.54 <sup>a</sup>	4.44 <sup>a</sup>
3%ginger	3.59	3.92	3.43 <sup>c</sup>	3.91 <sup>bcde</sup>	3.85 <sup>abc</sup>
5%ginger	3.40	3.65	3.02 <sup>c</sup>	3.45 <sup>e</sup>	3.44 <sup>c</sup>
1%garlic	3.67	3.96	4.41 <sup>ab</sup>	4.34 <sup>ab</sup>	4.24 <sup>ab</sup>
3% garlic	3.59	3.98	4.32 <sup>ab</sup>	4.24 <sup>abc</sup>	4.13 <sup>ab</sup>
5% garlic	3.63	3.99	4.21 <sup>ab</sup>	4.24 <sup>abc</sup>	3.99 <sup>abc</sup>
1%mixture	3.77	4.10	4.05 <sup>a</sup>	4.07 <sup>abcd</sup>	4.20 <sup>ab</sup>
3% mixture	3.23	3.72	3.22 <sup>c</sup>	3.55 <sup>de</sup>	3.66 <sup>bc</sup>
5% mixture	3.59	3.88	3.49 <sup>c</sup>	3.79 <sup>cde</sup>	3.68 <sup>bc</sup>

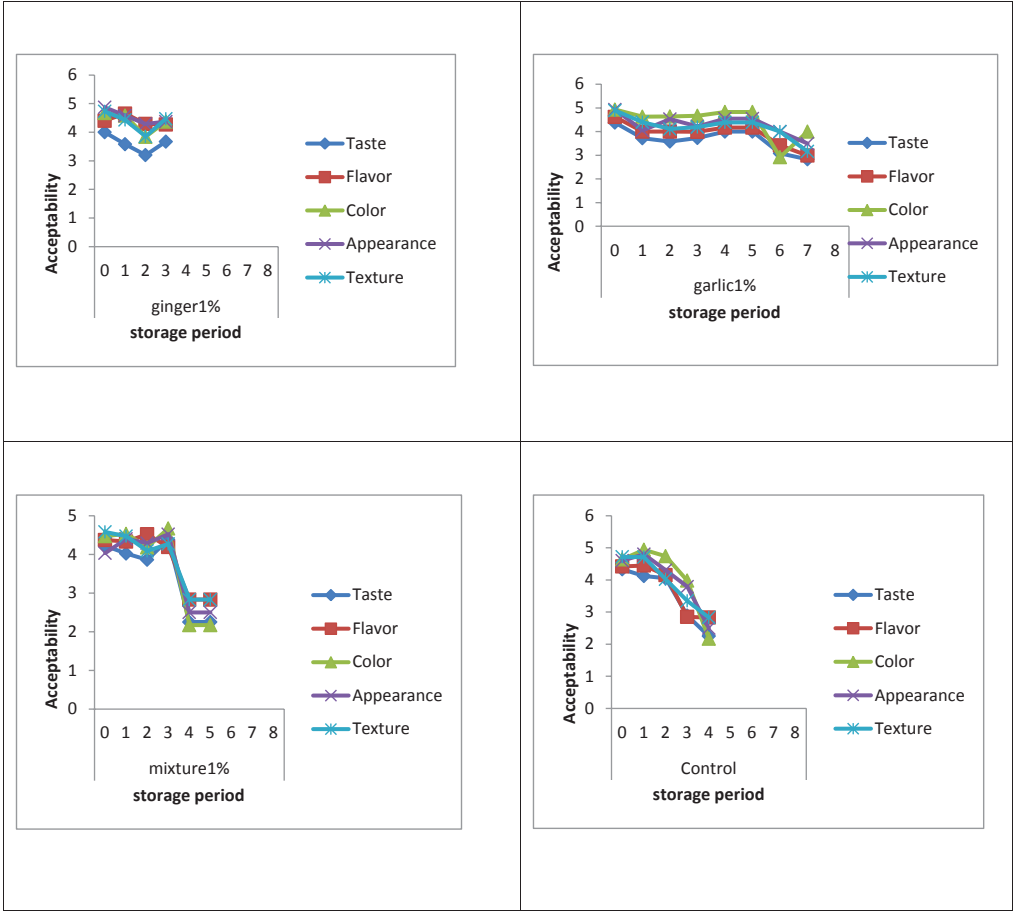
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#### 4.3.1. Organoleptic acceptability change during storage period

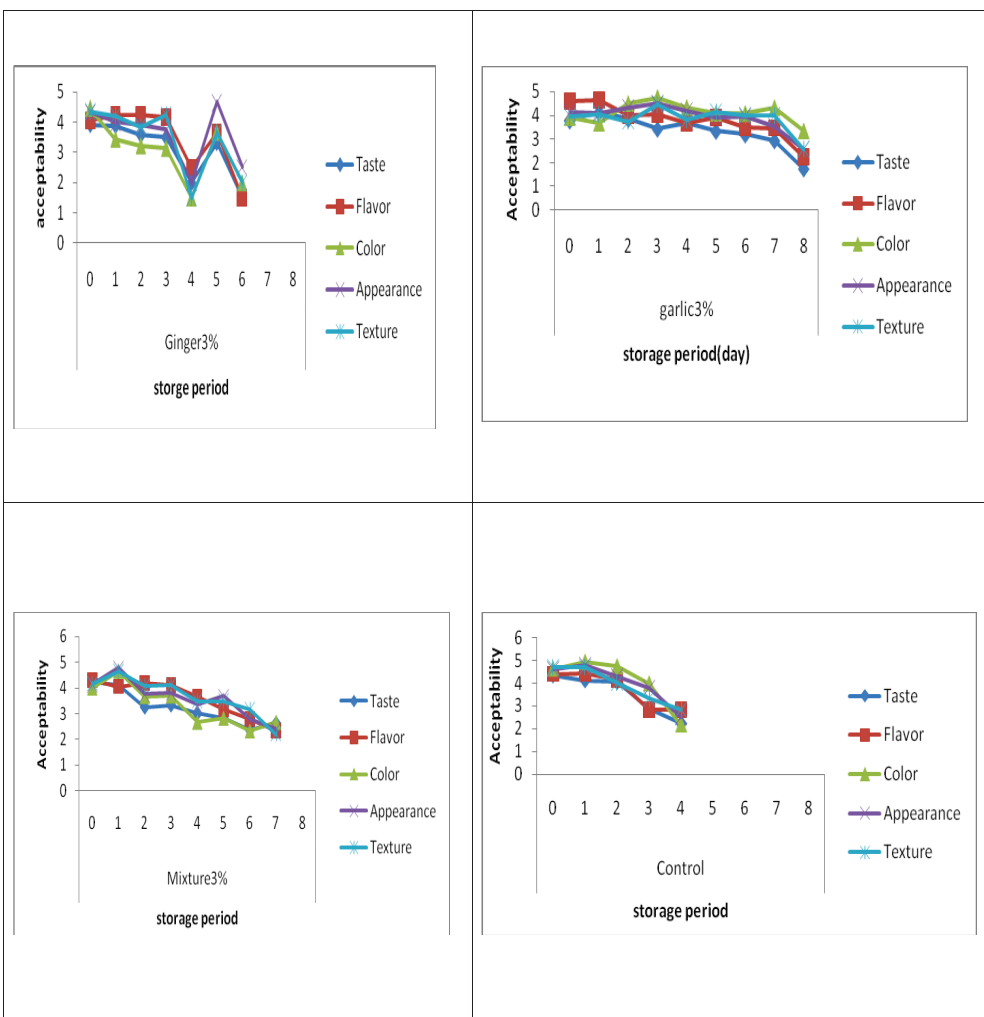
As the storage period advanced the organoleptic acceptability of the *Ayib* samples by the panelists tended to significantly ( $p<0.001$ ) decline (Figure 6, 7 and 8). This might

be due to the microbial activity in the *Ayib* samples that in turn might have affected the sensory score of the samples given by the panelists. Gundogdu *et al.* (2009) reported a similar result where sensory properties of yoghurt samples treated with garlic decreased during the storage period. The sensory scores given by the panelists to the *Ayib* samples treated with 1% ginger powder tended to decline down to the 2<sup>nd</sup> storage day then after inclined up throughout the storage period. One % garlic treated *Ayib* samples showed a declining sensory score throughout the storage period with the exception of color that was given an increasing score from 6<sup>th</sup> till the storage period. One % mixture treated *Ayib* showed a declining sensory score till 4<sup>th</sup> day then after a constant score towards end of the storage periods. The acceptability score of *Ayib* samples treated with 3% ginger powder decreased up to 4<sup>th</sup> day; on 5<sup>th</sup> day the score increased and then after a declining score was observed. *Ayib* treated with 3% garlic showed a declining value of taste, flavor and texture till 1<sup>st</sup> day while the color and appearance score showed an inclining score up to 2<sup>nd</sup> day; then after a declining score was observed. Three % mixture powder showed a declining sensory score of taste, flavor and color throughout the storage period while the texture and appearance showed an inclining score till 1<sup>st</sup> day then a declining score was observed. Five % ginger powder treated *Ayib* taste score showed an increasing score on 1<sup>st</sup> storage day while flavor and color showed a declining score till 2<sup>nd</sup> day, then the flavor inclining on the 3<sup>rd</sup> day and also the color showed an inclining score till storage day while the taste, appearance and texture showed a declining score starting from 2<sup>nd</sup> day till storage days. Five % garlic powder treated *Ayib* flavor showed a declining value on the 3<sup>rd</sup> and 5<sup>th</sup> day, the color showed a declining value on the 1<sup>st</sup>, 4<sup>th</sup> and 6<sup>th</sup> day, the texture on the 1<sup>st</sup> and 4<sup>th</sup> day, the appearance and also the taste showed a declining trend throughout the storage period. Five % mixture powder treated *Ayib* flavor, appearance and texture showed a declining value throughout the storage period, while

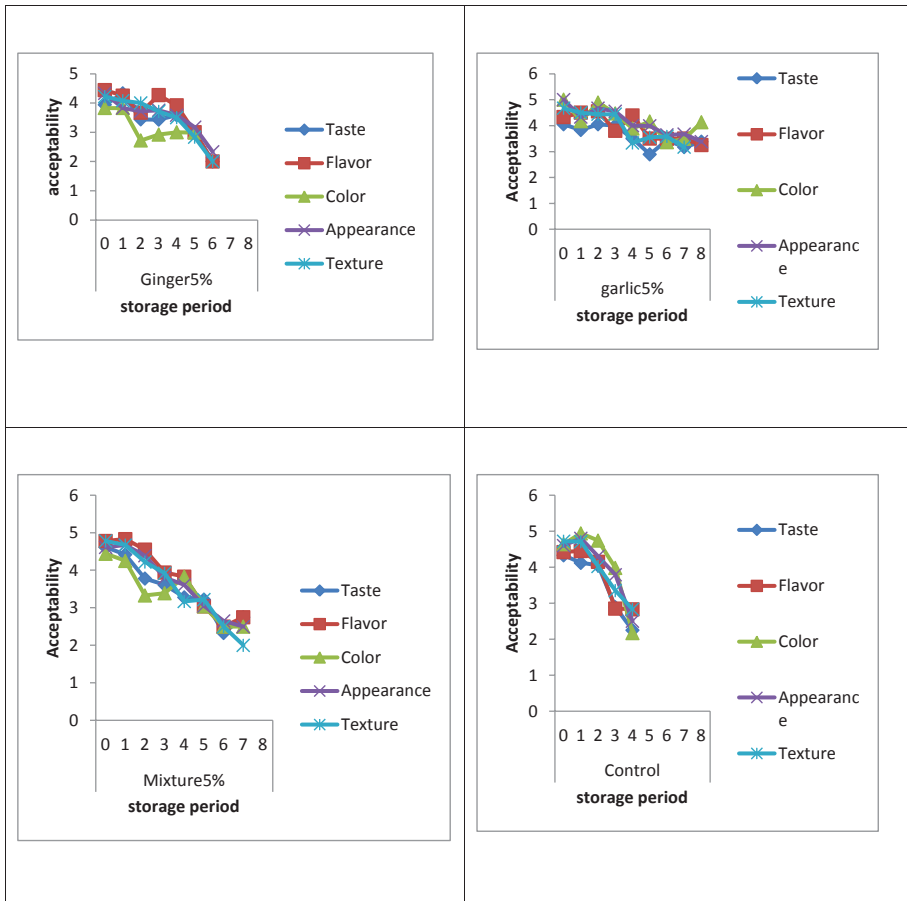
the color and taste showed an incling value on the 3<sup>rd</sup> and 4<sup>th</sup> day, then declining till the storage period.



**Figure 6.** Effect of ginger, garlic and their 1:1 mixture powder treated with 1% inclusion on acceptability during the storage period.



**Figure 7.** Effect of ginger, garlic and their 1:1 mixture powder treated with 3% inclusion on acceptability throughout storage period.



**Figure 8.** Effect of ginger, garlic and their 1:1 mixture powder treated with 5% inclusion on acceptability during the storage period.



Storage period showed marked ( $p<0.05$ ) effect on the organoleptic properties and CC of untreated *Ayib* samples (Appendix Table 6). As indicated by EL Owni and Hamid (2008), this might be due to the effect of proteolytic agents on the protein that can contribute for textural change and off-flavor through the breakdown of the released proteolytic products as amino acids, peptides into amines and acids. CC showed marked ( $p<0.05$ ) effect on the texture of untreated *Ayib* samples (Appendix Table 5). Storage temperature also had significant ( $p<0.01$ ) correlation with AMBC of untreated *Ayib* samples (Appendix Table 6). This might be due to storage temperature, which is favorable for aerobic mesophilic bacterial growth.

The ginger powder inclusion level had a significant ( $p<0.01$ ) negative correlation with color, appearance and texture of the *Ayib* samples (Appendix Table 7). This might be due to the color as well as fiber content of ginger powder that could have contributed to the color, appearance and texture change of *Ayib* samples. The storage period had a significant ( $p<0.001$ ) negative correlation with taste, aroma, color, appearance, texture, and coliform, and yeast and mould counts of ginger powder treated *Ayib* samples (Appendix table 7).

The garlic powder inclusion level had a significant ( $p<0.05$ ) negative correlation with aerobic mesophilic bacteria count of the *Ayib* samples (Appendix Table 8). This might be explained to the high antimicrobial activity of garlic powder inclusion where the activity might also increase with increasing level inclusion. The storage period had a significant ( $p<0.001$ ) negative correlation with taste, aroma, color, appearance and texture of garlic powder treated *Ayib* samples (Appendix table 8). Storage temperature also correlated significantly ( $p<0.05$ ) with the taste and aroma of garlic powder treated *Ayib* samples (Appendix table 8). The pH of garlic powder treated *Ayib*

samples showed significant ( $p<0.05$ ) correlation with the inclusion level and storage period (Appendix Table 8).

The storage period showed significant ( $p<0.001$ ) effect on organoleptic properties of the *Ayib* samples treated with mixture (1:1) powder (Appendix Table 9). The storage temperature also had significant ( $p<0.05$ ) correlation with aerobic mesophilic and yeast and mould counts of mixture powder treated *Ayib* samples (Appendix Table 9). This might be due to the moderate storage temperature that enhances their growth as they can grow even below at pH value 5.0 (Choisy *et al.*, 1986). The pH of mixture powder treated *Ayib* samples had significant ( $p<0.05$ ) correlation with inclusion level and CC (Appendix Table 9).

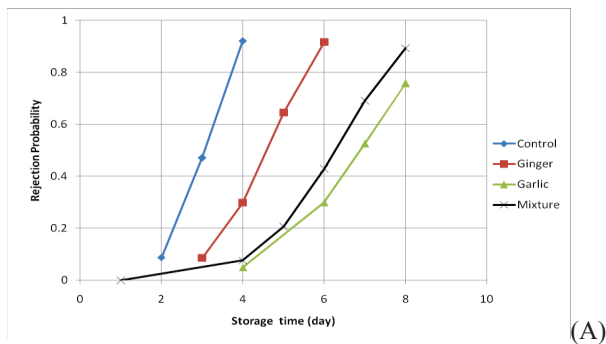
#### **4.4. Effects of ginger and garlic powder on shelf-life of *Ayib***

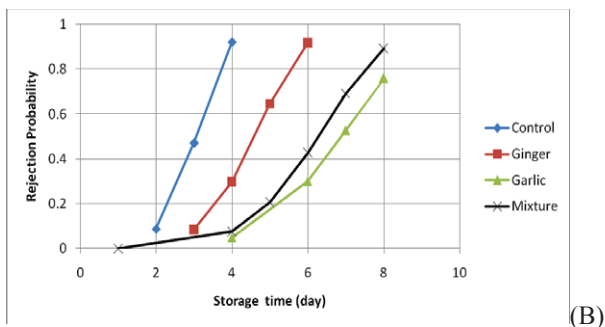
The shelf life of *Ayib* is determined based on organoleptic acceptability, when 50% of the panelists reject the product and the microbial shelf-life which bases the microbiological standards set for a quality Cottage cheese.

Treatments had significant effects ( $p<0.001$ ) on failure days of *Ayib* samples based on coliform and sensory shelf-life. However, the effects of treatments on Aerobic mesophilic and yeast and mould counts and shelf-life estimates were not significant.

According to the failure plot of sensory shelf life results, the highest failure rate was observed in untreated *Ayib* samples, while the lowest failure rate was observed in those treated with garlic powder (Figure 9). Based on the sensory failure day estimation, the shelf life of *Ayib* treated with garlic powder of 1% was 6.33, 3% was 8 and 5% was 7.66 days; while that of ginger powder treated with 1% was 3.67, 3% was

4.33 and 5% was 4.66 days; and that of mixture powder treated with 1% was 4.66, 3% was 7 and 5% was 7.33 days. The untreated *Ayib* had a shelf-life of 3.44 days. Based on bacteriological failure date estimation, the shelf life of *Ayib* treated with ginger and garlic powder was similar with that of sensory shelf life estimated day. Among the treatments, 1% ginger powder treated *Ayib* showed the highest failure rate, while 3% garlic powder treated *Ayib* the lowest. This might be due to the antimicrobial property of garlic as stated by Javed *et al.*, (2011) that might have hindered the microbial growth and their activity causing the unacceptable sensory and appearance properties of *Ayib* during the storage periods. This result is in agreement with that of Biniyam (2008), who reported that the shelf life of garlic juice treated cottage cheese showed the lowest failure rate than rue and garlic-rue mixture treated cottage cheese. However, the current failure rate of garlic powder treated *Ayib* was slower than garlic juice treated one. This might be due to the garlic powder that has more antimicrobial property than garlic juice. In addition, the storage temperature of garlic juice treated cottage cheese samples was warmer than the garlic powder treated *Ayib* samples.





**Figure 9.** Estimated cumulative Weibull distribution (A) for sensory expiry day and (B) for coliform count.

#### 4.5. Consumer Acceptability

The mean values of taste, aroma, color, texture and overall appearance of the *Ayib* samples ranged from 3.50 to 4.95, 3.77 to 4.86, 3.20 to 4.95, 3.80 to 4.91 and 3.43 to 4.86, respectively (Table 8). The consumer acceptability of the *Ayib* samples was highly affected ( $p < 0.05$ ) by the type and level of spice powder inclusion (Table 8). Untreated *Ayib* samples had the highest taste acceptability score among all the samples considered (Table 8). However, *Ayib* samples treated with 1 and 5% garlic, and 1% mixture powder didn't have significant acceptance score with untreated samples. One % garlic powder treated *Ayib* samples had highest consumer acceptability following untreated samples (Table 8). Untreated *Ayib* samples had the highest aroma acceptability score among all the samples considered (Table 8). However, *Ayib* samples with 1, 3 and 5% garlic, and 1% mixture powder inclusion didn't have significant acceptance score with untreated samples. Five % garlic powder treated *Ayib* samples got highest consumer acceptability following untreated samples (Table 8). Color of untreated *Ayib* samples had the highest acceptability score among the *Ayib* samples (Table 8). Although, the untreated *Ayib* samples had the highest

score, *Ayib* with 1 and 5% garlic, and 1% mixture powder didn't differ significantly in color score with untreated *Ayib* samples and 1% garlic powder treated *Ayib* samples received the second acceptability following the untreated one (Table 8). Untreated *Ayib* samples had the highest texture score among all the samples. However, 5% garlic and 1% mixture treated *Ayib* samples didn't have marked difference in texture acceptance score with untreated *Ayib* samples. One % mixture powder treated *Ayib* samples had the second score acceptability following untreated *Ayib* samples (Table 8). The mean value of overall appearance of untreated *Ayib* samples showed the highest score (Table 8). As it is the case with texture, 1 and 5% garlic and 1% mixture (1:1) powder treated *Ayib* samples didn't differ significantly with the untreated ones with 5% garlic treated *Ayib* samples having the second important overall appearance score following untreated *Ayib* samples (Table 8).

**Table 8.** Effect of ginger, garlic and their mixture on consumer acceptability (Mean  $\pm$  SE) of *Ayib* samples

Treatment	Taste	Aroma	Color	Texture	Appearance
Control	4.95 <sup>a</sup>	4.86 <sup>a</sup>	4.95 <sup>a</sup>	4.91 <sup>a</sup>	4.86 <sup>a</sup>
1%ginger	4.03 <sup>cde</sup>	3.94 <sup>cd</sup>	3.97 <sup>cd</sup>	4.19 <sup>bc</sup>	3.87 <sup>de</sup>
3%ginger	4.05 <sup>cde</sup>	4.14 <sup>bcd</sup>	3.73 <sup>de</sup>	3.95 <sup>c</sup>	3.95 <sup>cde</sup>
5%ginger	4.14 <sup>bcd</sup>	4.00 <sup>bcd</sup>	3.95 <sup>cd</sup>	3.95 <sup>c</sup>	3.90 <sup>cde</sup>
1%garlic	4.70 <sup>ab</sup>	4.30 <sup>abcd</sup>	4.70 <sup>ab</sup>	4.33 <sup>cb</sup>	4.30 <sup>bcd</sup>
3% garlic	4.32 <sup>bcd</sup>	4.29 <sup>abcd</sup>	4.36 <sup>cb</sup>	4.14 <sup>bc</sup>	4.25 <sup>bcd</sup>
5% garlic	4.48 <sup>abcd</sup>	4.57 <sup>ab</sup>	4.61 <sup>ab</sup>	4.57 <sup>ab</sup>	4.61 <sup>ab</sup>
1%mixture	4.62 <sup>abc</sup>	4.52 <sup>abc</sup>	4.52 <sup>abc</sup>	4.62 <sup>ab</sup>	4.48 <sup>abc</sup>
3%mixture	3.97 <sup>de</sup>	4.07 <sup>bcd</sup>	3.43 <sup>de</sup>	4.13 <sup>bc</sup>	4.03 <sup>cd</sup>
5%mixture	3.50 <sup>e</sup>	3.77 <sup>d</sup>	3.20 <sup>e</sup>	3.80 <sup>c</sup>	3.43 <sup>c</sup>

Means with similar superscript in a column are not significantly different (P>0.05)

The overall consumer acceptability mean values ranged from 4.91 to 3.54 (Table 9). The overall consumer acceptability mean value of untreated *Ayib* samples had the highest score (Table 9). However, *Ayib* samples with 1 and 5% garlic, and 1% mixture powder didn't have significant acceptance score with untreated samples. Five % garlic treated *Ayib* samples having the second overall acceptability score following untreated *Ayib* samples. This result is in line with that of Gundogdu *et al.* (2009) who revealed that yoghurt samples containing 1% garlic were more favored than samples with 0.5% in both set type and stirred type. *Ayib* samples treated with 5% mixture powder received statistically significant least score means among all the samples tested. The mean whiteness values of the *Ayib* samples ranged from 2.59 to 4.76 (Table 9). Untreated *Ayib* samples got the highest score on whiteness acceptability. However, *Ayib* treated with 1, 3 and 5% garlic and 1% mixture powder didn't have significant acceptance score with untreated samples. Five % garlic treated *Ayib* samples had the second whiteness acceptability score following untreated *Ayib* samples. The least whiteness acceptability value was recorded for the 5% mixture (1:1) powder inclusion.

**Table 9.** Overall consumer acceptability of (Mean  $\pm$  SE) of *Ayib* samples

<b>Treatment</b>	<b>Overall acceptance</b>	<b>Whiteness</b>
Control	4.91 <sup>a</sup>	4.76 <sup>a</sup>
1%ginger	4.00 <sup>cd</sup>	3.57 <sup>bc</sup>
3%ginger	3.96 <sup>cd</sup>	3.62 <sup>bc</sup>
5%ginger	3.99 <sup>cd</sup>	3.22 <sup>cd</sup>
1%garlic	4.47 <sup>ab</sup>	4.14 <sup>ab</sup>
3% garlic	4.27 <sup>bc</sup>	4.15 <sup>ab</sup>
5% garlic	4.57 <sup>ab</sup>	4.52 <sup>a</sup>
1%mixture	4.55 <sup>ab</sup>	4.19 <sup>ab</sup>
3% mixture	3.93 <sup>cd</sup>	3.05 <sup>cd</sup>
5% mixture	3.54 <sup>d</sup>	2.59 <sup>d</sup>

Means with similar superscript letters in a column are not significantly different

( $P>0.05$ )

## 5. SUMMARY, CONCLUSIONS AND RECCOMADATION

### 5.1. SUMMARY, CONCLUSIONS

The effect of ginger, garlic and their mixture powder at 1, 3 and 5% inclusion on consumer acceptability; organoleptic properties; microbial qualities; and physico-chemical properties of *Ayib* - Ethiopian cottage cheese during a storage period was studied.

Treating *Ayib* with ginger, garlic and their mixture powder did not affect the total solid, ash and fat contents throughout the storage period. The storage period affect the pH value of *Ayib*. The pH value of *Ayib* treated with (1 and 5%) ginger powder, and 1% garlic powder decreased up to day 2, while that treated with 5% garlic and 5% mixture powder decreased up to day 3 and 4, respectively, then after the pH of all the samples tended to increase till end of the storage period. The titratable acidity of *Ayib* was not affected by ginger, garlic and their mixture powder treatment during the storage periods.

Treating *Ayib* with ginger, garlic and their mixture powder did not show significant effect on AMBC, YMC and CC. *Ayib* treated with ginger and garlic powder qualify the global maximum values of cottage cheese ( $10^5$  cfu/g ) CC.

The organoleptic acceptability showed a declining trend as the storage period advanced. The Weibull analysis of the bacteriological failure rate in untreated samples was highest and garlic powder treated *Ayib* showed the lowest among all the samples.

Sensory failure rate in untreated samples was also highest, while garlic treated *Ayib* samples showed the lowest.

Untreated *Ayib* samples had the highest acceptability score among all the samples considered. However, *Ayib* samples with 1 and 5% garlic, and 1% mixture powder didn't have significant acceptance score with untreated samples. The consumer acceptability of *Ayib* treated with 5% garlic powder showed the highest result as compared with all the treated *Ayib* and the whiteness acceptability of untreated *Ayib* had the highest score followed by 5% garlic treated had the second score with 5% mixture treated *Ayib* samples showing the lowest score.

Therefore, from the current study it can be concluded that treating *Ayib* with garlic powder significantly decreases the coliform count and improves the shelf life of the product without markedly affecting its % total solid, ash and fat contents, while keeping its consumer acceptability.

## **5.2. RECOMMENDATION**

Inclusion of 3% garlic powder in *Ayib* can significantly decrease the coliform counts and prolong its shelf life without affecting its composition.

Further investigation on the identification, isolation and appropriate inclusion level of active ingredients of garlic powder that inhibit the dominant microbes responsible for *Ayib* spoilage is needed.

A study on traditionally used spices and herbs that have a potential to improve *Ayib* shelf life together with the appropriate processing method and sensory acceptable inclusion level is required. There is also a need to undertake more research work to isolate and characterize the dominant microbes that are responsible for *Ayib* spoilage.



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## 7. APPENDICES

**Appendix Table 1.** ANOVA for proximate composition of *Ayib* samples

Variable	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Total solid	Treatment	9	11.608473	1.289830	0.16	0.9957
	Error	18	142.953046	7.941835		
	Corrected Total	29	196.436377			
	C.V	10.35				
Ash	Treatment	9	0.054401	0.006044	0.90	0.5440
	Error	18	0.120706	0.006706		
	Corrected Total	29	0.583236			
	C.V	6.11				
Fat	Treatment	9	0.414332	0.046037	1.09	0.4171
	Error	18	0.761394	0.042299		
	Corrected Total	29	2.720221			
	C.V	15.23				

**Appendix Table 2.** ANOVA for pH and titratable acidity of *Ayib* samples

Variable	Source	DF	Sum of square	Mean Square	F Value	Pr > F
pH	Treatment	9	0.093289	0.010366	0.92	0.5291
	Error	18	0.202418	0.011245		
	Corrected Total	29	0.326264			
	C.V	2.47				
Titratable Acidity	Treatment	9	0.038736	0.004304	0.16	0.9957
	Error	18	0.477621	0.026534		
	Corrected Total	29	0.929197			
	C.V	21.80				

**Appendix Table 3.** ANOVA for microbial count ( $\log_{10}$  cfu/g) of *Ayib*

Variable	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Aerobic mesophilic	Treatment	9	2.068878	0.229875	2.16	0.0785
	Error	18	1.915483	0.106416		
	Corrected Total	29	10.297928			
	C.V	4.65				
Coliform	Treatment	9	6.906992	0.767444	1.89	0.1204
	Error	18	7.322995	0.406833		
	Corrected Total	29	19.889071			
	C.V	30.74				
Yeast and Mould	Treatment	9	8.053468	0.894829	1.03	0.4528
	Error	18	15.607126	0.867062		
	Corrected Total	29	27.397459			
	C.V	12.36				

**Appendix Table 4.** ANOVA for microbial count (log<sub>10</sub> cfu/g) of *Ayib* during the storage period

Variable	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Aerobic mesophilic Bacteria	Treat	9	14.497532	1.610837	2.02	0.0422
	Day	8	14.941532	1.867692	2.34	0.0222
	day*treat	57	30.517907	0.535402	0.67	0.9536
	Model	76	103.394250	1.360451	1.71	0.0040
	Error	125	99.637016	0.797096		
	Corrected	201	203.031266			
	Total					
	C.V	12.69				
	Treat	9	52.175754	5.797306	2.55	0.0100
	Day	8	127.664633	15.958079	7.03	<.0001
Coliform	day*treat	57	121.403030	2.129878	0.94	0.5999
	Model	76	338.879760	4.458944	1.96	0.0004
	Error	125	283.831928	2.270655		
	Corrected	201	622.711689			
	Total					
	C.V	71.78				
	Treat	9	48.818897	5.424322	1.19	0.3091
Yeast and Mould	Day	8	41.769366	5.221171	1.14	0.3398
	day*treat	57	186.876349	3.278532	0.72	0.9200
	Model	76	337.273819	4.437813	0.97	0.5501
	Error	125	571.308392	4.570467		
	Corrected	201	908.582212			
	Total					
	C.V	28.503				

**Appendix Table 5.** ANOVA panelist acceptability of *Ayib* during the storage period

Variable	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Taste	Treatment	9	8.806361	0.978485	1.71	0.0883
	Error	196	112.002798	0.571443		
	Corrected Total	205	120.809159			
	C.V	20.88				
Flavor	Treatment	9	6.456419	0.717379	1.13	0.3402
	Error	196	123.947593	0.632386		
	Corrected Total	205	130.404012			
	C.V	20.09				
Color	Treatment	9	63.852788	7.094754	11.72	<.0001
	Error	196	118.689648	0.605559		
	Corrected Total	205	182.542435			
	C.V	19.69				
Appearance	Treatment	9	23.876556	2.652951	4.70	<.0001
	Error	196	110.727735	0.564937		
	Corrected Total	205	134.604291			
	C.V	18.58				
Texture	Treatment	9	17.384126	1.931569	2.80	0.0042
	Error	196	135.386808	0.690749		
	Corrected Total	205	152.770934			
	C.V	20.89				

**Appendix Table 6.** Pearson correlation of level, storage day, taste, flavor, color, appearance, texture, aerobic mesophilic, coliform, yeast and mould, storage temperature and pH of untreated *Ayib* during the storage period.

C	level	Day	Taste	aroma	color	App	texture	amb	coli	ymc
level	1.0000									
	0.1383	1.0000								
day	0.4581									
	0.0169	-0.5003	1.0000							
taste	0.9280	0.0041								
	-0.2108	-0.5019	0.7632	1.0000						
aroma	0.2550	0.0040	<.0001							
	-0.0410	-0.2602	0.6452	0.6786	1.0000					
color	0.8266	0.1573	<.0001	<.0001						
	0.0327	-0.4686	0.7741	0.7462	0.8621	1.0000				
appear	0.8613	0.0078	<.0001	<.0001	<.0001					
	-0.0226	-0.5700	0.6451	0.6765	0.7971	0.8961	1.0000			
texture	0.9036	0.0008	<.0001	<.0001	<.0001	<.0001				
	-0.1451	-0.0851	0.2577	0.2100	0.2303	0.3286	0.3280	1.0000		
amb	0.4360	0.6488	0.1615	0.2566	0.2126	0.0711	0.0716			
	-0.1743	-0.4099	0.2711	0.3029	0.0789	0.2862	0.3566	0.4370	1.0000	
coli	0.3481	0.0220	0.1401	0.0976	0.6729	0.1185	0.0489	0.0140		
	-0.2497	-0.1026	0.3103	0.4411	0.2573	0.2874	0.2750	0.6708	0.4633	1.0000
ymc	0.1754	0.5828	0.0893	0.0130	0.1622	0.1169	0.1343	<.0001	0.0087	
	0.0811	-0.0200	0.2556	0.3364	0.1690	0.1504	0.2212	-0.4779	-0.0523	-0.1490
temp	0.6642	0.9148	0.1651	0.0642	0.3634	0.4190	0.2317	0.0065	0.7796	0.4235
	0.2422	0.0182	-0.1591	-0.1271	-0.1596	-0.0603	0.0737	0.0324	0.15341	0.0646
pH	0.1892	0.9225	0.3924	0.4955	0.3909	0.7471	0.6933	0.8624	0.4100	0.7299

C= control, app= appearance, amb= aerobic mesophilic, coli= coliform, ymc= yeast and mould, temp= temperature



**Appendix Table 7.** Pearson correlation of the level, storage day, taste, flavor, color, appearance, texture, aerobic mesophilic, coliform, yeast and mould. storage temperature and pH of ginger powder treated *Ayib* during the storage period.

g	Level	day	Taste	aroma	color	appear	texture	Amb	coli	ymc	temp	pH
level	1.00000											
	0.2425	1.0000										
day	0.1266											
	-0.0060	-	1.0000									
		0.6315										
taste	0.9703	<.0001										
	-0.2821	-	0.7065	1.0000								
		0.6641										
aroma	0.0739	<.0001	<.0001									
	-0.4863	-	0.5380	0.6781	1.0000							
		0.5539										
color	0.0013	0.0002	0.0003	<.0001								
	-0.4178	-	0.5936	0.7816	0.8978	1.0000						
		0.5775										
appear	0.0066	<.0001	<.0001	<.0001	<.0001							
	-0.3644	-	0.7228	0.8428	0.7558	0.8459	1.0000					
		0.7139										
texture	0.0191	<.0001	<.0001	<.0001	<.0001	<.0001						
	-0.0909	0.0547	-	-	0.0580	-	-	1.0000				
			0.2927	0.1890		0.1233	0.0767					
amb	0.5718	0.7341	0.0633	0.2364	0.7185	0.4422	0.6332					
	0.2946	-	-	-	-	-	-	0.4764	1.0000			
		0.0378	0.0943	0.2614	0.0498	0.1301	0.1525					
coli	0.0615	0.8144	0.5574	0.0986	0.7572	0.4173	0.3409	0.0016				
	-0.1572	-	-	-	0.1895	-	-	0.8525	0.29183	1.0000		
		0.0252	0.1298	0.0394		0.0006	0.0082					
ymc	0.3260	0.8753	0.4186	0.8067	0.2354	0.9968	0.9591	<.0001	0.0641			
	-0.0276	-	0.0976	0.1621	0.1058	0.1966	0.0674	-	-0.0371	-	1.0000	
		0.0923						0.3804		0.2678		
temp	0.8637	0.5658	0.5435	0.3110	0.5100	0.2178	0.6750	0.0141	0.8179	0.0904		
	-0.0645	-	0.2367	0.2271	-	0.0719	0.1578	-	-0.0033	-	0.2746	1.0000
		0.1474			0.0007			0.0852		0.0384		
pH	0.6885	0.3577	0.1362	0.1533	0.9963	0.6550	0.3242	0.5960	0.9832	0.8113	0.0822	

g= ginger, app= appearance, amb= aerobicsophilic, coli= coliform, ymc= yeast and mould, temp= temperature

**Appendix Table 8.** Pearson correlation of the level, storage day, taste, flavor, color, appearance, texture, aerobic mesophilic, coliform, yeast and mould, storage temperature and pH of garlic powder treated *Ayib* during the storage period.

r	Level	Day	Taste	aroma	color	appear	texture	amb	coli	ymc
level	1.0000									
day	0.1370	1.0000								
	0.2546									
taste	-0.0106	-0.5116	1.0000							
	0.9299	<.0001								
aroma	0.0276	-0.6005	0.7534	1.00000						
	0.8190	<.0001	<.0001							
color	-0.1067	-0.4567	0.5204	0.4322	1.0000					
	0.3758	<.0001	<.0001	0.0002						
appear	-0.0519	-0.5924	0.6689	0.6137	0.6832	1.0000				
	0.6668	<.0001	<.0001	<.0001	<.0001					
texture	-0.1494	-0.5045	0.6500	0.5888	0.5577	0.7373	1.0000			
	0.2136	<.0001	<.0001	<.0001	<.0001	<.0001				
amb	-0.2390	-0.0667	-0.0946	-0.0007	-0.0232	-0.0276	-0.0571	1.0000		
	0.0447	0.5802	0.4322	0.9954	0.8475	0.8192	0.6361			
coli	-0.0412	-0.2228	0.0009	0.0662	0.0111	0.1732	0.09067	0.1690	1.0000	
	0.7328	0.0617	0.9938	0.5833	0.9267	0.1485	0.4520	0.1588		
ymc	-0.1514	-0.1201	-0.0498	0.1154	-0.0574	0.0656	-0.0612	0.7541	0.3101	1.0000
	0.2074	0.3182	0.6799	0.3377	0.6341	0.5867	0.6121	<.0001	0.0085	
temp	0.1429	0.0168	0.3041	0.2311	0.08608	0.0917	0.14402	-0.1092	-0.0284	-0.0756
	0.2344	0.8891	0.0099	0.0524	0.4754	0.4468	0.2308	0.3643	0.8136	0.5306
pH	0.2700	0.2475	-0.1336	-0.1119	-0.0250	-0.0227	-0.0627	0.1656	0.0979	0.1575
	0.0228	0.0374	0.2665	0.3525	0.8360	0.8504	0.6030	0.1674	0.4165	0.1895

r= garlic, app= appearance, amb= aerobicmesophilic, coli= coliform, ymc= yeast and mould, temp= temperature

**Appendix Table 9.** Pearson correlation of the level, storage day, taste, flavor, color, appearance, texture, aerobic mesophilic, coliform, yeast and mould, storage temperature and pH of mixture (1:1) treated *Ayib* during the storage period.

m	Level	day	Taste	aroma	color	appear	texture	Amb	coli	Ymc	temp	pH
level	1.0000											
	0.0820											
day	0.2303	1.0000										
	0.0820											
taste	-0.0928	-	1.0000									
	0.4883	0.7516										
	<.0001											
aroma	-0.0880	-	0.7577	1.0000								
	0.7456											
	<.0001											
color	0.5109	<.0001	<.0001									
	-0.2304	-	0.7504	0.7998	1.0000							
	0.6618											
	<.0001											
appear	0.0818	<.0001	<.0001	<.0001								
	-0.1068	-	0.7743	0.8732	0.7883	1.0000						
	0.6795											
	0.4245	<.0001	<.0001	<.0001	<.0001							
texture	-0.1720	-	0.7137	0.8119	0.7456	0.8585	1.0000					
	0.7108											
	<.0001											
amb	0.1965	<.0001	<.0001	<.0001	<.0001	<.0001						
	-0.0623	0.0281	-0.0482	-0.0994	-0.0041	-0.1482	-	1.0000				
	0.1398						0.2950					
	0.6418	0.8341	0.7193	0.4576	0.9756	0.2667	-					
coli	0.0594	-	0.0561	-0.1051	0.0565	-0.0416	-	0.4110	1.0000			
	0.0883						0.0379					
	0.6577	0.5096	0.6754	0.4320	0.6731	0.7560	0.7771	0.0014				
ymc	-0.0951	0.0363	-0.1108	-0.1008	-0.0118	-0.1210	-	0.7757	0.4134	1.0000		
	0.1051						0.4322					
	0.4775	0.7865	0.4076	0.4515	0.9299	0.3652	<.0001	0.0013				
temp	0.1379	0.1684	0.0165	-0.0759	-0.0702	-0.0725	-	0.2796	0.0424	0.2786	1.0000	
	0.0709						0.5966					
	0.3019	0.2062	0.9020	0.5710	0.6004	0.5884	0.0335	0.7519	0.0341			
pH	0.2628	0.1232	0.0212	-0.1056	-0.0413	-0.0769	-	0.2274	0.3872	0.0018	0.0706	1.0000
	0.1845						0.1655	0.0859	0.0027	0.9891	0.5984	

m= mixture, app= appearance, amb= aerobicsesophilic, coli= coliform, ymc= yeast and mould, temp= temperature

**Appendix Table 10.** ANOVA for consumer acceptability of *Ayib* samples

Variable	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Taste	Treat	9	43.110449	4.790049	5.28	<.0001
	Error	248	225.013582	0.907313		
	Corrected Total	257	268.124031			
	C.V	22.42				
Aroma	Treat	9	24.654287	2.739365	3.02	0.0019
	Error	248	225.190674	0.9080269		
	Corrected Total	257	249.844961			
	C.V	22.59				
Color	Treat	9	80.111341	8.901260	9.91	<.0001
	Error	248	222.849899	0.898588		
	Corrected Total	257	302.961240			
	C.V	23.07				
Texture	Treat	9	25.570498	2.841166	3.60	0.0003
	Error	248	195.530278	0.7884285		
	Corrected Total	257	221.100775			
	C.V	20.94				
Appearance	Treat	9	39.538205	4.393134	5.15	<.0001
	Error	248	211.438539	0.852575		
	Corrected Total	257	250.976744			
	C.V	22.30				

**Appendix Table 11.** ANOVA for overall consumer and whiteness acceptability of *Ayib*

Variable	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Overall acceptability	Treat	9	37.691457	4.187939	6.84	<.0001
	Error	248	151.886217	0.612444		
	Corrected Total	257	189.577674			
	C.V	18.67				
Whiteness	Treatment	9	90.333336	10.037037	8.95	<.0001
	Error	197	220.884054	1.121238		
	Corrected Total	206	311.217391			
	C.V	27.99				

7.2. SENSORY EVALUATION SCORE SHEET

Date.....

In this sensory evaluation you will conduct:

1. Acceptability test

You will be given *Ayib* samples. Write the sample code in the blank spaces and kindly evaluate the products for 5 = like very much, 4 = like moderately, 3 = neither like nor dislike, 2 = dislike moderately, 1 = dislike very much. Please don't forget to rinse your mouth between samples and at the end.

Please provide the following information:

Gender:            M   ☐            F   ☐

Age:  

Sample Code / No.....

Sensory Perception	Quality Attributes				
	Taste	Aroma	Colour	Texture	Appearance
Like very much					
Like moderately					
Neither like nor dislike					
Dislike moderately					
Dislike very much					

Additional comment.....

Thank you very much for your time and contribution!

7.3. SENSORY EVALUATION SHEET FOR WHITENESS

Date.....

You will be given *Ayib* samples. Use the scales to indicate your attitude by checking at the point which best describes your feeling about the *Ayib*.

Please provide the following information:

Gender     M ☐                      F ☐

Age:

Code	Acceptance	Scale	Sensory Attributes
			Whiteness of the <i>Ayib</i>
	Very white	5	
	Moderately white	4	
	White	3	
	Less White	2	
	Not White	1	

Comments.....

Thank you very much for your time and contribution!

## **BIOGRAPHICAL SKETCH**

The author was born in 1978 at Debre Zeit. She attended her elementary and high school in Angelis School and Bishoftu High school, respectively at Deber Zeit, Oromia Region.

She then joined the then Awassa College of Agriculture in 1997 and graduated with a B.Sc. degree in Animal Production and Rangeland Management in July 2000. Thereafter, she joined Unity University from October 2000 to June 2001 to attend Accounting. Following that the author was employed in Assella Technical and Vocational Education Training College as instructor and served for 8 years and transferred to Adama Science and Technology University since 2009 as instructor. She then joined the School of Graduate Studies of Harmaya University in October 2009 for her post-graduate studies in the field of Food Science.







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