

**ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
FACULTY OF SCIENCE
FOOD SCIENCE AND NUTRITION PROGRAM**



**INVESTIGATION ON NUTRITIONAL AND MICROBIOLOGICAL
PROPERTIES OF ABYSSINIAN DONKEY MILK FROM ADEA
WOREDA**

**BY
TIRINGO TADESSE**

**A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES
OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE
REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN
FOOD SCIENCE AND NUTRITION**

**JUNE, 2010
ADDIS ABABA**

**INVESTIGATION ON NUTRITIONAL AND MICROBIOLOGICAL
PROPERTIES OF ABYSSINIAN DONKEY MILK FROM ADEA WOREDA**

BY

TIRINGO TADESSE

**Advisors: Professor Nigusse Retta
Dr. Alemayehu Lemma
Dr. Genene Tefera**

JUNE, 2010

Addis Ababa

ACKNOWLEDGMENTS

Preparation of this study has been a collaborative effort of different advisors and colleagues. First and foremost, I would particularly wish to acknowledge my advisors Prof. Niguse Retta, Dr Alemayehu Lemma and Dr Genene Tefera who took the lead in revising my research topic, commenting, re-writing and editing the entire paper. My special thanks also goes to Dr. Belay Tefera who contributed his valuable time in providing professional inputs, comments and guidance in all aspects. I also wish to thank w/o Almaz Gonfa for her all professional assistance. This paper also would not have been possible without the generous support of people in the study area and owners of donkeys in facilitating and organizing sample collection process namely Mr, Yilma Dire, Mesfin Teklue and Biruk Aragaw. I would also like to thank Melkassa Agricultural Institute, especially Mr, Mulugeta Teamir. I would like to express my gratitude to Mr, Delnesaw Zerfu, Ayele Degefa, Yonas Gebrehiwot and Abel Anberbir and Institute of biodiversity and to Chemical engineering department laboratory staffs particularly Yosan Teshome, Biruk Yohanis and biruk Tefera. Last but not least, I would like to extend my gratitude to my husband-Dhaba Fayissa for his effort in editing, encouraging and empowering me in all aspects of my work.

Table of Contents

ACKNOWLEDGEMENTS.....	II
List of Tables.....	IV
List of Figures.....	V
List of Abbreviations and Acronyms.....	VI
ABSTRACT.....	VII

Chapter - 1

INTRODUCTION.....	1
--------------------------	----------

Chapter – 2

LITERATURE REVIEW.....	6
2.1. Milk production from donkeys.....	7
2.1.1. Values of donkey’s milk.....	8
2.1.2. Yield and quality of donkey milk.....	10
2.1.3. Production of donkey milk in the world.....	11
2.2. Milk production from other non bovine mammals.....	12
2.3. Potentials of donkey milk production in Ethiopia.....	13
2.4. Properties of donkey milk.....	14
2.4.1. Fermenting activity.....	14
2.4.2. Antimicrobial activity.....	15
2.4.3. Protein composition.....	17
2.4.4. Other properties of donkey milk.....	19
2.5. Nutritional characteristics of donkey milk.....	21
2.5.1. Donkey’s milk total protein.....	21
2.5.2. Nutritional sufficiency of donkey milk.....	23
2.5.3. Use of donkey milk as human milk substitutes.....	24

Chapter – 3

MATERIALS AND METHODS.....	27
3.1. Study area and study animals.....	28
3.2. Sample collection	29
3.2.1. Sample collection.....	29
3.4. Instrument and methodology	30

3.4.1. <i>Physical analysis</i>	31
3.4.2. <i>Proximate analysis</i>	31
3.4.3. <i>Mineral analysis</i>	33
3.4.4. <i>Microbiological analysis</i>	36
5. Statistical Analysis.....	38
Chapter – 4	
RESULTS AND DISCUSSION	39
4.1 Physical analysis.....	40
4.2 Proximate analysis.....	41
4.3 Mineral analysis.....	43
4.4. Factors affecting milk composition.....	45
4.5. Microbiological analysis	46
Chapter – 5	
CONCLUSION AND RECOMMENDATIONS	50
Chapter – 6	
REFERENCES	52
APPENDEIX	59

List of Tables

Table 1. Physicochemical composition of donkey and human milk value obtained from literature.....	10
Table 2. Human, Livestock and donkey population in Garaboru, Babodaya and Hiddi.....	27
Table 3. Analysis methods used for the quantification of components of Donkey milk	29
Table 4. Working vitamin C standard preparation	32
Table 5. Working standards for Phosphorus determination	34
Table 6: physical properties of Abyssinian donkey milk samples collected from Garaboru, Babodaya and Hiddi.....	39
Table 7. Chemical composition of Abyssinian donkey milk samples collected from Garaboru, Babodaya and Hiddi	41
Table 8. Mean (\pm SD) of mineral composition of Abyssinian donkey milk samples collected from Garaboru, Babodaya and Hiddi	42
Table 9. Test of differences between contents of Abyssinian donkey milk in the present study and literature values for human milk and donkey milk	43
Table 10: Differences in physicochemical parameters and composition of Abyssinian donkey milk contents among the three study sites.....	44
Table 11. Mean value of microbial count for TAC, GN bacteria, G.P positive, Yeast and mold in log CFU/g of donkey milk Samples collected from the three sampling sites.....	46
Table 12. Bacterial strains isolated from Abyssinia donkey milk that were suspected as Lactic acid	47

List of Figures

Figure 1-Standard curve for ascorbic acid determination.....	32
Figure 2-Standard curve for phosphorous determination.....	34
Figure 3- Change in microbial flora of Abyssinian donkey milk samples during storage for 18 days.....	46

List of Abbreviations and Acronyms

CFU/ML	Colony Forming Unit per Mili liters
Diplo	Diplococcic
gn.Lacto or Entro	genus Lactobacillus or Entrococcus,
gn.Lb	genus Lactobacillus
AOAC	Association of Official Analytical Chemistry
CMA	Cow milk allergy
CSA	Central Statistics Agency
DCC	Differential leukocyte Cell Count
EHNRI	Ethiopian Health and Nutrition Research Institution
FAO	Food and Agriculture Organization
GN	Gram negative
Gp	Gram positive
Gm-rxn	Gram-reaction
IDF	International dairy federation
IS	International standard organization
LAB	Lactic acid bacteria
LPS	Lactoperoxidase system
MRS	Mann Rogussa Sharp
PA	Peasant association
PCA	Plate count agar
PDA	Potato dextrose agar
APP	Acute phase proteins
SCC	Somatic Cell Count
UNICEF	United Nation International Children's Fund
UNRRA	United Nations Relief and Rehabilitation Administration
UV	Ultraviolet

ABSTRACT

A study was carried out on donkey milk in Adea woreda with particular emphasis on characterization of the nutritional and microbiological properties of Abyssinian donkey milk from Adea woreda. The results were compared with those already published in literature for a potential future use as replacement of human breast milk. To this end, the study utilized primary data that were collected through direct collection of milk from 24 Jennies from three selected kebeles of Ada'a woreda; and secondary data were obtained from published literature for comparative purposes. Attempts were primarily made to analyze the physicochemical composition such as fat, total protein, lactose, minerals, vitamins C, acidity or pH, density, total solid and freezing points; and microbiological characteristics. Results indicated that the Abyssinian donkey milk have close similarity with other donkeys milk previously investigated elsewhere for most of the components. More importantly, the present findings confirmed Abyssinian donkey milk to have a very close similarity with human breast milk for protein, lactose, vitamin C, density, and acidity contents. However, it was found to have a significantly higher ($p < 0.01$) concentration in all minerals investigated in this study than human breast milk. With regards to the microbiological properties, it was found out that the donkey milk didn't ferment, and had lower microbial load as is common in cow's milk. The present findings can generally provide strong evidence that the Abyssinian donkey milk in Adea woreda can provide an alternative supplement for human breast milk in many aspects of its composition.

Key words: Abyssinian donkey, milk, nutritional content, microbiological property

Appendix 1. Picture of the study area and some activities



Figure 1 Gara boru area where Donkey milk samples are collected



Figure – 2 Hidi area/PA where Donkey milk samples are collected



Figure 3: Sample collection from Abyssinian donkey



Figure 4: Block digestion system for protein determination



Figure 5: Distillation system for protein determination

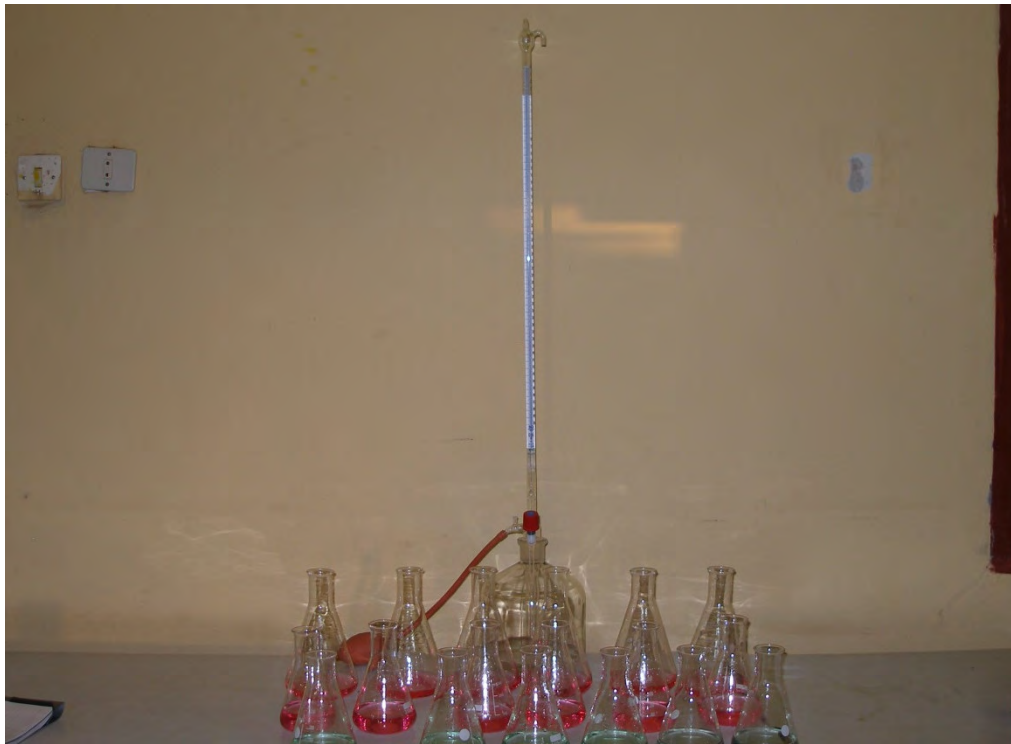


Figure 6 Titration process with 0.1 N HCL

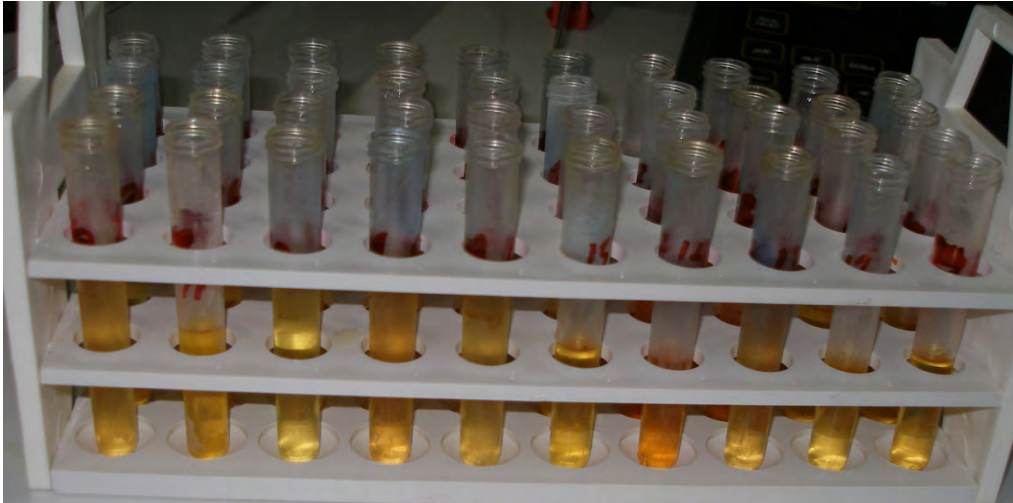


Figure 7: Color development for ascorbic acid determination



Figure 8: Complex formation for phosphorus determination



Figure 9: Wet digestion system



Figure 10: Samples with sterile bottle



Figure 11: Media Preparation

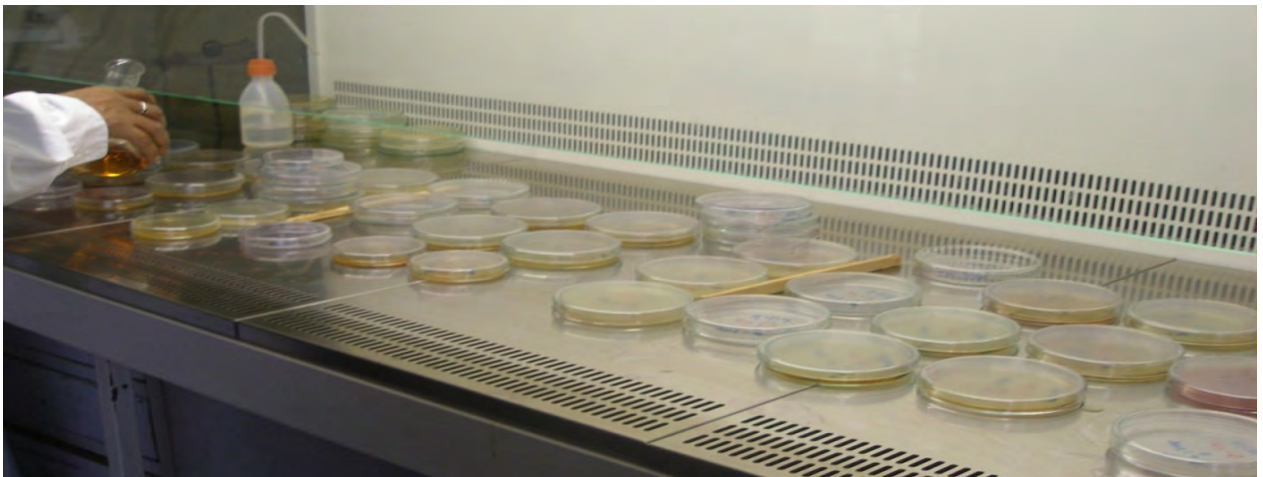


Figure 12: Solidification of media after inoculation

Chapter - One

INTRODUCTION

Ethiopia holds the largest livestock population in Africa; which is estimated (excluding some regional stat) at about 43.1 million heads of cattle, 23.6 million of sheep, 18.6 million of goats, 0.62 million of camels, 34.2 million of chicken, 2.8 million of horses, 6.5 million of donkeys, 0.3 million of mules (CSA, 2008). However, milk production is among the lowest in the world; 1.54 liters (per cow per day) milk production from dairy cows (CSA, 2008). The contribution of the different livestock species to the total production of milk is about 81.2% from cattle, 6.3 % from camels, 7.9 % from goats and 4.6 % from ewes (CSA, 2008). The per capita milk consumption (16 kg/year), reported (Saxena *et al.*, 1997) has not changed much to date and is much lower than reports for Africa (27 kg /year) and developed world (100 kg/year), respectively. This clearly depicts the presence of a wide gap between the demand and supply of milk and milk products.

The dairy industry is not developed even when compared with east African countries like Kenya, Uganda and Tanzania. Regarding dairy production, the national milk production remains among the lowest in the world, even by African standards (Zegeye, 2003). The demand for dairy products is increasing as ever. Ethiopia is not self-sufficient in milk and a considerable amount of foreign exchange has to be spent on the import of dairy products. In Ethiopia one of the leading causes of infant mortality is malnutrition. Although milk is considered to be nature's perfect food for the growing infant, the ever rising scarcity and high cost of milk and dairying has made it impossible to meet the demand. Due to the highly perishable nature of milk and miss handling, the amount produced is subjected to high post-harvest losses. Losses of up to 20–35 % have been reported in Ethiopia for milk and dairy products from milking to consumption (Getachew, 2003). Although the dairy cow has been the predominant domesticated animal species for dairy production in developed countries, goats, sheep, water buffalo, yak, camel, and mare as well as some other minor mammalian species in which donkey is the one, have been domesticated and bred for milk production in regions of the world where the difficult environment required special adaptation and for which many of the non bovine mammals are better suited. While non bovine animals are an integral part of mixed-farming systems and hold several roles in Ethiopia, they provide the most needed animal power for the agriculture in addition to their production of milk and meat (Young, 2006).

The donkey is an important livestock species that is well adapted to the different environments. It serves for riding, as a pack animal for short distance transport, and as a draft

animal. A study showed that owning a donkey reduces the work load of women and at the same time increases time spent on other income generating activities (Ezedeen, 1998). A donkey gives about two liters of milk per day, over three milking, while a cow can churn out 40 liters in a single milking (Stephen *et al.*, 2006).

Donkey milk has long been praised for its unique nutritional quality and its closest similarity to human milk (Carroccio *et al.*, 2000). Consequently, donkey milk has been recently considered as a substitute for cow's milk protein due to its tolerance and nutritional adequacy in children with cow milk protein allergy and as safe, natural food in the condition where breastfeeding is not possible (Tesse *et al.*, 2009). Moreover, different studies also confirm donkey milk to be a natural product with good dietary and cosmetic properties (Carroccio *et al.*, 2000), used for probiotic purposes (Coppola *et al.*, 2002), with good palatability and optimization of the intestinal absorption of calcium which is essential for bone mineralization in infants (Schaafsma, 2003), and has high levels of linoleic and linolenic acid which are useful in the treatment of atopic dermatitis, tuberculosis and malaria (Horrobin, 2000). It has also a protective nature against viral fevers and flu-like symptoms such as asthma, runny nose and cough (Horrobin, 2000). However, there are no objective studies carried out to characterize the content and microbiological properties of donkey milk in Ethiopia for a potential use as safe and natural human breast milk supplement, substitute or its medical value.

Despite the presence of enormous products of animal origin, most are not consumed due to lack of awareness, religious, cultural taboos, and virtual absence of objective researches to bring neglected foods to human consumption. Moreover, women and children face huge and complex nutritional problems. One-fourth of Ethiopian women are malnourished, and approximately 50% of children less than five years of age are moderately or severely stunted (CSA, 2006). Analysis shows that malnutrition, even in its milder forms, accounts directly or indirectly for 53% of under-five deaths in Ethiopia (Burns, 2001). It is well known that the best nutritional option for newborns is their mother's milk, however, some infants may not be exclusively breast fed during the first months of life.

Accordingly, to alleviate the problem of infant mortality it is very important to improve optimal infant and young child feeding practices (Burns, 2001). While commercial milk replacements have become totally inaccessible due to cost, there is a need for another

substitute such as donkey milk which is claimed to be similar in composition and properties as human milk. This kind of research could pave the way to promote and raise awareness of the society on the importance and benefits of donkey milk and to break the social stigma associated with it. Moreover, this study might be the first of its kind in Ethiopia which would serve as a breakthrough in combating taboo against the donkey milk. Milk contents, on the other hand vary based on species, breed, locality, and stage of lactation. In order to use donkey milk for infant milk replacement, one needs to fully know the basic contents, and the biochemical characteristics. In this study it was hypothesized that donkey milk is equivalent in content and characteristics to human breast milk. Therefore, the general objective of the current study is to provide information about Abyssinian donkey milk from Adea Woreda that can potentially be used to replace or supplement human breast milk; while the specific objectives were:

- To characterize the nutritional content of Abyssinian donkey milk
- To analyze the microbiological properties of Abyssinian donkey milk

The significance of the study

The following are some of the significance of this study;

- This study could help as source of evidence and help create awareness to the community about donkey milk
- It will serve as an advocacy tool to minimize the negative attitude and taboo associated with donkey in general and donkey milk in particular
- The study also has add value for donkeys beyond the known value accustomed by society such as transportation, pack, riding and draft animal.
- The result of this investigation will help to show that donkey as an alternative or optional means for infant nutrition
- The finding of the study will have an importance as a substitution in helping children who are allergic to cow's milk protein. This is because donkey milk has close similarity with mother's milk which in all cases has no allergy to children
- It can serve as a starting or entry point for other researchers to investigate further on nutritional, medicinal and microbiological and cosmetic values of donkey milk
- Even though Ethiopia has the largest population of donkeys in Africa, this large resource has not been properly utilized. So, this study is hoped to pave a way for appropriate utilization of donkey's resource for milk and other purposes.

Limitation of the Study

This study has focused on Abyssinia donkeys which are mainly concentrated in the central parts of the county. So, this study is delimited only to the aforesaid types of donkeys and geographically to Adea woreda in Oromia region. In addition, the negative attitude and taboo associated to donkey milk hampered the researcher to get as many data as possible since owners are not willing to allow their donkeys for milking.

Other limitation of this study is scarcity of milk from donkeys. Given that most of the donkeys have not got enough and appropriate hay or food, it was a challenge to collect enough milk from sampled population at limited period of time and took several days in collecting milks for this purpose. Getting information about sampled donkeys was also another challenge since most of the owners don't have reliable information about their donkey's back history. This also limits the researcher to give full classifications of sample population.

Farmers or owners expectation for incentives from the researcher was also another area which can be considered as a limitation.

Chapter - 2

LITERATURE REVIEW

2.1. Milk production from donkeys

Donkeys are kept in Africa for four reasons: work, breeding, milking and eating. Of these, work is most important, and used mainly as pack animals, either for carrying loads or for riding. In arid regions they are used together with camels to pull water from deep wells. Less commonly they are used in traction, for example, pulling carts or plows, although both of these technologies are post-European introductions in sub-Saharan Africa (Kathy and Zahra, 2000).

Donkey's milk has been used for long period of time in the history of human kind. Dating back in 2500 B.C, Egyptians used this milk for different purposes. However, the first real scientific consideration was not given to this exceptional milk until the Renaissance period (Stephen *et al.*, 2006). One of the interesting historic report shows that Cleopatra, queen of Egypt, maintained her great beauty by bathing in donkey's milk and in same author report, the Romans also used donkey's milk for its therapeutic qualities. Like Cleopatra of Egypt, Poppaea, the wife of the emperor Nero, used this precious liquid to maintain the health and beauty of her skin (Stephen *et al.*, 2006). As indicated by Stephen and his colleagues, with the advice of doctors, François 1st king of France who was exhausted by wars underwent donkey's milk treatment and he found it very incredible and relieved him from stress and fatigue.

In the 19th century men and women of fashion from upper status society regularly consumed this precious beverage, but poor families saved this miraculous substance for a sick child or a weakened old person only. During this time, donkey's milk started to be regularly used in maternity hospitals and used to feed new-born (Tesse *et al.*, 2009). Furthermore, until the beginning of the 20th century, donkey's milk was marketed for the feeding of orphan babies, unhealthy children, ill people and the elderly. Tesse and colleagues, (2009) further elaborated that in the tradition of this use of donkey's milk, farms of donkeys were set up in Italy, France, Belgium, Switzerland and Germany during the start of 20th century. The success of these special farms that exploit donkey's milk confirms the product's ancestral reputation and all the significance of its vital properties. The modern day scientific community highly acknowledges that the value and importance of this heritage.

2.1.1. Values of donkey's milk

Donkey's milk is a milk which is rich in vitamins and has essential fatty acids and protein very similar to human mother's milk and it can be recommended in case of allergies to cow's, ewes and soya milk or simply when the mother wishes to give her child a safe, natural food (Tesse *et al.*, 2009). Similarly, donkey milk shares much similarity with mare milk in being low in total solids (8 to 10 %), protein (1.5 to 1.8 %) and being high in lactose (6 to 7 %). Its protein percentage has been characterized as having low casein nitrogen (47.3 %) as well as a high concentration of essential amino acids such as Val and Ly (Taha and Kielwein, 1990). Whey protein contents (36.9 %), where β -Lacto-globulin (LG) accounted for just 29.85 % (Salimei *et al.*, 2004).

As mentioned above when compared with milk from other species, donkey's milk presents a low dry material, fat and protein content but it is rich in lactose and calcium (Taha and Kielwein, 1990). The high lactose content of donkey's milk is revealed to be quite similar to that of a human's milk. This plays a fundamental role since it stimulates the intestinal absorption of calcium by having a positive influence on bone mineralization and since it stimulates the correct development of the nervous system in a child's first few months (Taha and Kielwein, 1990).

The level of fat in donkey milk ranges from 0.28 % (Chiavari *et al.*, 2005) to 1.82% (Ofstedal and Jenness, 1988). The lipid fraction of donkey's milk presents high levels of essential fatty acids. A fatty acid is qualified as "essential" when the organism can only produce them in small quantities. It is recommendable if human being in general and children in particular are provided with these essential acids throughout their daily diet or in the form of nutritional supplements, depending on type of the case (Guo, *et al.*, 2007). Some research findings depicts that the essential fatty acids present significant curative advantages as they contribute to the development of the brain and the retina. It is richer in long chain polyunsaturated fatty acids that also predominate in women's milk (52-54%), with a high percentage of linoleic acid (essential polyunsaturated fatty acid not synthesized by the organism); these polyunsaturated fatty acids seem to have immune-stimulant properties and cholesterol-lowering agents (Taha and Kielwein, 1990). Omegas 3 contained in donkey's milk play a major role with regard to the health of human

heart, since they help to fight against thrombosis (forming of blood clots) and heart rhythm disorders seem to be of precious help in preventing risks following a cardiac accident (Taha and Kielwein, 1990).

Different research reports show that donkey's milk has other importance apart from its nutritional value. It also presents a number of interesting characteristics in cosmetology (Vincenzetti *et al.*, 2007) because of its vitamins (A, B2, C and E) content and the vitamins in donkey milk enable to have several anti-ageing and skin regeneration effects which play an anti-oxidant role (Guo, *et al.*, 2007). Donkey milk has high vitamin C and in turn, vitamin C plays an anti-oxidizing role much sought after in cosmetics. It thus favors' the slowing down of the ageing process and speeds up the healing mechanisms. Vitamin E is recognized as being essentially an anti-oxidizer that slows down skin ageing and ensures the stability of cell structures (Iacono, *et al.*, 1992).

According to Iacono and colleagues, (1992) findings, it also contain a lot of unsaturated fatty acids such as Omegas 6 that are highly prized in cosmetics and contains a sufficient quantity of nourishing lipids to ensure the flexibility and well-being of the skin. Consequently the same authors found out that essential fatty acids which is rich in donkey's milk helps the skin to absorb vitamins as it allows the skin to become elastic and prevent certain skin diseases (Iacono, *et al.*, 1992). Mineral salts contained in donkey's milk allow the skin to be cleaned. They get rid of dead cells, thus, leaving room for cells that live on the skin's surface and influence cell growth (Vincenzetti *et al.*, 2007).

Another most important and interesting part of donkey's milk consumption for human being is its hygienic value. Its pH is close to neutrality (7.2), but higher than that of cow's milk (pH = 6.3to 6.8). Its strong lysozyme content and its relative shortage of casein and phosphate explain, in all likelihood, the low microbial contamination of donkey's milk (Zhang *et al.*, 2008). Indeed, the quantity of bacteria found is very low: 4.46 log cfu/ml. These values only increase very little at stages of advanced lactation: 5.2 log cfu/ml. The rate of maximum bacteria tolerated by international directives for human milk consumption is not an entry barrier, far from it. This amount even represents an asset for donkey's milk in the context of industrial processing of a microbiologically sensitive product. Fresh donkey's milk represents ideal nourishment in the

context of minimum risk food. The high lysozyme content, which has a selective action vis-à-vis potentially pathogenic micro flora in the gastrointestinal tract of the new-born. The high lysozyme content of donkey's milk makes this food naturally "sterile" with an extremely low bacterial burden even after being kept in the fridge for 15 days with no preventive heat treatment (Zhang *et al.*, 2008).

Table 1. Physicochemical composition of donkey and human milk obtained from literature

Milk characteristic	Donkey milk			Human milk		
	N	Mean	SD	N	Mean	SD
PH	70	7.18	0.13	20	6.89	0.08
Density	150	1.03	0.02	20	1.027	0.011
Acidity%	-	-	-	20	0.072	0.011
TSS %	150	8.91	0.31	20	10.71	0.11
Fat %	70	0.38	0.12	20	2.1	0.15
Protein %	70	1.56	0.14	20	1.94	0.14
Lactose %	70	6.33	0.17	20	6.45	0.08
Vitamin C	-	-	-	26	3.06	0.67
Calcium	24	676.7	284.32	20	340	3.5
Magnesium	24	58.46	8.43	20	30	0.35
Iron	24	1.15	0.51	20	0.4	0.00
Zinc	24	1.22	0.52	20	2.3	0.16
Phosphors	6	487.0	-	20	160	0.85

Source: Elisabetta *et al.*, 2009; Dorea, 2000; Salimei *et al.*, 2004; Guo, *et al.*, 20007

2.1.2. Yield and quality of donkey milk

The small cisternal size, the structure of mammary gland in Equidae affects the need for a greater number of daily milking (Doreau and Boulot, 1989). The asinine species is considered a seasonal polyestrous one, but the latitude in which the farm is located can greatly influence the reproduction cycle; considering a recent investigation in the south of Italy, specifically in Sicily, the local latitude determined small photoperiod oscillations between the different seasons, and under these conditions the donkeys have a continuous reproductive cycle, the same was observed

also in sheep (Giosuè *et al.*, 2008). In Sicily it is in fact possible to obtain donkey milk throughout the year by adequately planning the breeding seasons; results showed that donkeys foaling in winter and in summer produced more milk than those foaling in the other seasons. Considering the different milk yield obtained when donkeys were milked twice a day, in the morning and in the afternoon (Salimei *et al.*, 2004), the average milk yield of the morning milking was found to be statistically lower than that observed for the afternoon milking, that is 549.2 mL vs. 949.3 mL respectively (D'Alessandro and Martemucci , 2007) have investigated on the effects of daily milking number and frequency on donkey milk production and the results obtained in that study demonstrated that the highest milk yield corresponded to three milking per day every three hours, while daily milking regimen of six milking per day did not increase milk production and had a negative influence on the health of the mammary gland.

2.1.3 Production of donkey milk in the world

Even though all mammals produce milk, it is only few species which produce milk that is utilized by human beings. The most important mammals which produce milk are cattle, buffalo, sheep, goat, horse, donkey and camel. The quantities of milk produced by various species differ markedly and individual animals also show wide variations in production within one species, mainly depending on domestic purpose, breed and genetic quality, environmental conditions, physiological conditions and level of management. Mares, donkeys and camels are principally kept as draught, pack or riding animals. Milk production is secondary and milk is only incidentally consumed, although in some areas the milk of these animals is highly appreciated and may be used for the manufacture of special fermented milk (Van Den Berg, 1988).

The advantages of donkey milk have attracted considerable interest in its use for human consumption today. However, donkey breeding is so disperse that the milk yield is very low. With almost 8 million head of donkey in 2004, China has the largest donkey stock worldwide, followed by Ethiopia (FAO, 2005). Donkeys are primarily raised in Northwest China in the Xinjiang and Shanxi provinces. In the last several years, the Xinjiang province has strived to establish donkey-breeding centers, leading to a great improvement in the yield of donkey milk.

Annual milk production has reached up to 40,000 metric tons, ranking first in world (Yang *et al.*, 2006), and has helped make donkey milk processing possible.

2.2. Milk production from other non bovine mammals

No one can deny the fact that cows are the primary dairy animal species in many countries to provide humans with nutritious food through the abundance of their lacteal secretion. Goats and other minor dairy species will never be able to compete with cows in terms of volume of milk production. However, the contribution of milk from other domesticated dairy species to the survival and well being of people around the world is immense and invaluable, especially in areas where cows have difficulty to surviving. Milk of non bovine animals is not only for people in the developing world who want and need to better their food supply quantitatively and qualitatively, but is especially for people in the developed world who may have medical needs for alternative foods and treatment, or for a higher- quality menu.

Sheep have been milked for millennia, but mainly as part of triple-purpose breeding for fiber and meat production besides milk. Therefore official statistical records of dairy sheep populations, and sheep milk production and processing, are hard to find. Sheep milk has unique composition and is ideally suited for yogurt and cheese production. Buffalo milk is important in Asian countries mainly, but the distribution of buffalo populations and interest in buffalo milk products is spreading. India has supported officially significant research with dairy buffaloes. Buffalo milk is popular in many traditional products, which are not well known in Western countries. Mare milk is another Asian uniqueness, with much tradition in some countries, but also with some good justification as an alternative to cow milk and treatment for humans with debilities. Camel milk also has unique compositional differences from cow milk, among which is an absence of beta-lacto globulin, which makes it more similar to human milk (Young, 2006).

Reindeer milk has received new interest from researchers in the North, mainly because of its highest level of milk composition and the unique adaptation of this ruminant mammal to the very harsh climate. Protein in reindeer milk is about three times the level in cow milk, but lactose is uniquely less. Sow milk is of considerable academic and research interest because of the

physiological similarity of this monogastric to human milk secretion. Nutritional, physiological, and biochemical research data on sow milk can be effectively utilized and applied to related situations in human metabolism, health, and medicine (Young, 2006).

Additional other minor species milks such as moose, musk, donkey, zebra, and pony are produced for human consumption only at certain specific regions of the world, very little data is available due to the extremely limited studies that have been conducted on such rarely domesticated minor species mammals. Academically, there should be much stimulation from the different uniqueness among those species (Young, 2006). The technical and popular literature abounds with publications about the world of cow milk, while milk of other mammals has given little attention. Non-bovine mammals have an important nutritional, economic, and social role. Non-bovine mammals includes: goats, sheep, buffaloes, mares, camels, donkey, yaks, reindeer, sows, llamas, and humans. Focus on donkey milk is the initial motivator for this paper, because of some unique characteristics of donkey milk compared to cow milk. Donkey milk has been used successfully in cases of cow milk allergies and by patients with various metabolic and gastrointestinal ailments. Donkey milk proteins can differ genetically from cow milk proteins (Carroccio, 1999).

2.3. Potential of donkey milk production in Ethiopia

The importance of donkeys in the past as well as to date has not been considered a significant component of the development process. Leave alone donkey's milk, beyond for the transportation purpose, donkey is considered one of the insignificant animal in Africa in general and in Ethiopia in particular. The attitude of the society towards donkey is not positive. For many of the institutions promoting 'development', the use of donkeys has been considered an indicator of backwardness and underdevelopment. Traditional attitudes to donkeys have also been quite negative and in some instances have inhibited the spread of donkey use. This attitude has led to a loss in the traditional knowledge relating to donkeys and to a lack of investment in the research and development of donkey issues. Field observations however indicate that this may be changing. In parts of Ethiopia, farmers observed that in periods of significant food insecurity, donkeys were more important than oxen (Kathy Marshall and Zahra Ali, 2000). The taboo and

negative attitude associated with donkey has led to neglect and less care. Now the importance of the donkey is finally being appreciated, a positive change in their image is possible. This should lead to a greater acceptance and awareness of this species as an important resource.

Since Ethiopia has the largest donkey population 6.5 million (CSA, 2008) and largest density in the world (27 donkeys per 100 people; Pearson *et al.*, 2006), it is possible to enhance donkey milk production by establishing donkey-breeding centers, which leads to an improvement in the yield of donkey milk. Considering its unique nutrient profile and economic potential, donkey milk could be exploited to fulfill the nutritional requirements of the people of Ethiopia particularly children. However, nothing is known about domestic donkey milk in Ethiopia. Therefore, there should be a need to promote the use of donkey milk in Ethiopia and to develop specialized center of donkey breeding particularly in those area where they are predominantly found and potentially productive.

2.4. Properties of donkey milk

2.4.1. Fermenting activity

The possibility of producing a fermented beverage from donkey's milk using the probiotic bacterial strains help to understand the microbiological properties of donkey milk which has a low microbiological load and an elevated content of lysozyme. The bacterial strains employed for fermentation had a good growth capacity in donkey's milk only after an initial adaptation phase. An extremely high percentage of viable bacteria were found in the final beverage. Likewise, the activity of lysozyme was virtually unchanged with respect to initial value. Technology can be proposed for production of a fermented beverage from donkey's milk that can be utilized by small producers. This would allow the production of a beverage that would be well accepted by consumers interested in a product with favorable therapeutic properties integrated with probiotic bacteria (Chiavari *et al.*, 2005).

Enzymes in donkey milk possess some unique characteristics such as bactericidal property which makes donkey milk different from other mammals' milks (Malacarne *et al.*, 2002). Milk is a good

medium for the growth of many microorganisms, since it contains many necessary nutrients and provides a suitable physical environment (White, 2001). In another way, milk supplies an array of defense factors in the form of lactoperoxidase peroxide system (LPS), lactoferrin, lysozyme, immunoglobulins and free fatty acids that protect it from different microbial infections.

2.4.2. Antimicrobial activity

Report from different professional in the area indicate that donkey milk contains several antimicrobial factors such as lactoferrin, lactoperoxidase and lysozyme, that have the ability to kill and inhibit a broad spectrum of bacteria in the digestive tract to reduce the incidence of gastro-intestinal infections or by defending against infections of the mammary gland. Milks of different species contain different amounts of the various antimicrobial factors.

Lactoferrin presents in milk are synthesized by the epithelial cells of the mammary gland. Human milk is particularly rich in lactoferrin. The concentration in the colostrums ranges from 1 to 16 g/l where as in the mature milk is 1-2 g/l. In bovine colostrums lactoferrin ranges from 0.2 to 5 g/l but decreases in mature milk to 0.1-0.2 g/l (Miranda *et al.* 2004; Greppi and Roncada, 2005), in donkey's milk the level of lactoferrin is reported to be two times higher than that of bovine milk (Malacarne *et al.*, 2002). Lactoferrin is an iron-binding protein and involve in the hydrolysis of glycosidic bonds of mucopolysaccharides in bacterial cell walls. Donkey milk is also known to contain a low microbial content (Chiavari, 2005; Coppola, 2002). This can be attributed to the antimicrobial substances present in donkey milk.

Other authors reported the donkey's milk has two variants of lysozyme that are lysozyme A and B that differ for three amino acid substitutions at different position (Herrouin *et al.* 2000). Lysozyme is an enzyme presents in the milk of some species, in human milk the concentration is 0.2 g/l, in bovine and in goat milk is nearly undetectable, in mare's milk is 1.1 g/l (Miranda *et al.*, 2004). In particular, the concentration of lysozyme in donkey milk reaches the level of 4 g/ L, (Guo *et al.*, 2007). The high lysozyme content of donkey milk may be responsible of the low bacterial count reported in literature and also makes this milk suitable to prevent intestine infection to infants.

In addition, lysozyme has other physiological functions, including inactivation of certain viruses, immuno- regulatory activity, anti-inflammatory and antitumor activity (Ibrahim & Aoki, 2003). Several studies have indicated that lysozyme derived from hen egg white, marine bacterium and shark cartilage possesses effective angiogenic inhibition and anti-tumor activities (Jiao *et al.*, 2004; Sava, 1989; Ye, Wang, *et al.*, 2008). The anti-tumour mechanism can be summarized into two different categories: enhancement of immunity against tumours (immuno-enhancing activity), through stimulating the production of immuno-enhancing and tumoricidal cytokines and direct anti-tumors activity to induce apoptosis of tumors cells (Zhang, *et al.*, 2006).

The active components of donkey milk had an accumulation effect on the G0/G1 and G2/M phases of A549 cells, i.e., the proliferation of A549 cells were arrested in the G0/G1 and G2/M phases of the cell cycle. This observation explains the anti proliferative effect of donkey milk. Several reports have shown that cell proliferation and differentiation were specifically controlled in the G1 phase (Hall & Peters, 1996). The significant suppressive effect of donkey milk on the proliferation of tumor cells is partly attributed to the promotion of differentiation of A549 cells into normal cells. According to Chihara (1992), donkey milk could induce a marked increase in cytokine production from lymphocytes and macrophages. These cytokines are contributory to an immune response and result in maturation, differentiation, and proliferation of immuno-competent cells for defense mechanisms of the host which explains the possible immuno-enhancing mechanism of the anti-tumour activity of donkey milk.

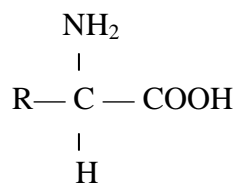
The concentration and activity of each antimicrobial system/substance show wide variation among species (Chandan *et al.*, 1965). Donkey milk is reported to have stronger inhibitory activity than that of any other species.

2.4.3. Protein composition

It is clearly known that proteins are indispensable for the growth, development and maintenance of all cells in the body. Various research outcomes found out that the value of milk proteins depends primarily on their content of some nine essential amino acids which cannot be

synthesized by the body. Cow and human milk contain all the essential amino acids required for human infants. Fortunately, both cow and human milk are easily digested and the amino acids are readily absorbed. Proteins are essential to all life process and perform a variety of functions in living organisms ranging from providing structure to reproduction. The natural function of milk proteins is to supply young mammals with the essential amino acids required for the development of muscular and other protein-containing tissues, and with a number of biologically active proteins, e.g. immunoglobulin's, vitamin-binding protein, metal-binding proteins and various protein hormones.

In the history of human kind, milk proteins represent one of the greatest contributions of milk to human nutrition. Proteins are polymers of amino acids that means only 20 different amino acids occur regularly in proteins. The amino acids have the following general structure (O'Connor C B, 1995).



The properties of the acid are affected by different radicals of amino acid and in this case each amino acid has different radicals. The content and sequence of amino acids in a protein affect its properties. Some proteins contain substances other than amino acids and are called conjugated proteins; these include Phospho-proteins in which phosphate is linked chemically to the protein, e.g. casein in milk and phosphoproteins in egg yolk. Lipoproteins which are combinations of lipid and protein are excellent emulsifying agents. They are found in milk and egg yolk. Chromo proteins which have a colored prosthetic group and include hemoglobin and myoglobin (O'Connor C B, 1995).

About 95% of nitrogen in milk is in the form of protein. Multiplication of the nitrogen content by a kjeldhal factor of 6.38 is generally accepted to give the protein content in milk and milk products, although the kjeldhal factor varies among milk protein (Pieter, 2006). Normal bovine milk contains about 3.5% protein (Weeb, *et al.*, 1974). The concentration changes significantly

during lactation, especially during the first few days post-partum; the greatest change occurs in the whey protein fraction. The young of different species are born at very different states of maturity, and, consequently, have different nutritional and physiological requirements. These differences are reflected in the protein content of the milk of the species; the protein content of milk is directly related to the growth rate of the young of that species, reflecting the requirements of protein for growth.

The properties of many dairy products, in fact their very existence, depend on the properties of milk proteins, although the fat, lactose and especially the salts, exert very significant modifying influences. Casein products are almost exclusively milk protein while the production of most cheese varieties is initiated through the specific modification of proteins by proteolytic enzymes or isoelectric precipitation. The high heat treatments to which many milk products are subjected are possible only because of the exceptionally high heat stability of the principal milk proteins (Van den Berg J C T. 1988). Specifications for many dairy products include a value for protein content. Changes in protein characteristics such as insolubility as a result of heat denaturation in milk powders or the increasing solubility of cheese proteins during ripening are industrially important features of these products.

Initially, it was believed that milk contained only one type of protein but about 100 years ago (Dairy Science and Technology, 2006) it was shown that the proteins in milk could be fractionated into two well-defined groups. On acidification to pH 4.6 (the isoelectric pH) at around 30°C, about 80% of the total protein in bovine milk precipitates out of solution; this fraction is now called casein. The protein which remains soluble under these conditions is referred to as whey or serum protein or non-casein nitrogen (FAO, 1990). The ratio of casein : whey proteins shows large interspecies differences; in human milk, the ratio is 40 : 60, in equine milk is 50: 50 while in the milks of the cow, goat, sheep and buffalo it is 80 : 20.

In addition to the caseins and whey proteins, milk contains two other groups of proteins the protease-peptone fraction and the non-protein nitrogen fraction. The heat-denaturable whey proteins represented the lacto globulin and lactalbumin fractions and designated the remaining 20% protease-peptone. The protease-peptone fraction was precipitated by 12% trichloroacetic acid

but some nitrogenous compounds remained soluble in 12% TCA and were designated as non protein nitrogen (FAO, 1990).

2.4.4. Other properties of donkey milk

Donkey milk hygiene should also be evaluated, as recently stated by Conte (2008). The EC Regulation n. 853/2004 allows the sale of ass' milk being these animals included in the "other milk producing species". According to this EC Regulation, the donkey milk should have a total bacterial plate count of less than <1,500,000/ml at 30°C. But, if this milk is assigned to infants or elderly human beings, it's absolutely necessary that, besides the nutritional requirements, the sanitary aspects will be evaluated too. Conte (2008) has already discussed the European indications as inadequate for the control of milk quality in donkeys, demonstrating that in presence of some milk bacteria (*S. aureus*, *S. intermedius*, *S. dysgalactiae*) there are no evidences, in donkeys, of clinical signs and/or increased somatic cell count (SCC). The dairy industry is currently focusing on SCC as marker of both milk quality and udder health, and the important role of SCC in the pathogenesis of mastitis is well described (Morgante *et al.*, 1996). Peeler *et al.* (2000) have demonstrated that sometimes, in and «infected» milk (in cows, goats and ewes), the changes does not regard only the number of cells but the type of them. Furthermore, as the severity of inflammation associated with the mammary gland disease increases, the protein composition of milk changes approaching that of blood (as a consequence of increased permeability of the blood mammary barrier or intramammary synthesis). Many of these proteins are the so called «acute phase proteins» (APP). In bibliography no data exist on the differential leukocyte cell count (DCC) for donkey milk, neither on the acute phase proteins (APP) fluctuations during an entire lactation period and/or during mastitis. The peculiarity of donkey milk together with the potential consumers require indeed an accurate evaluation of milk quality and udder health that should pass through the evaluation of DCC, together with the acute phase proteins (APP) assessments.

2.5. Nutritional characteristics of donkey milk

As being repeatedly mentioned above, milk is one of the most common causes of food allergies among children under one year of age. No specific therapy exists for this allergy, and thus the only feasible response is to avoid consumption of milk and derived products. Studies conducted on the serum of children with hypersensitivity to milk have shown that caseins are the proteins with the greater allergenic potential. However, in some cases, children have also shown hypersensitivity to the B-lactoglobulines and to the α -lactalbumins.

Various and dependable research reports show that when food intolerance is diagnosed in an infant, it is often necessary to impose a period of total parental feeding, followed by breast feeding, considered the most correct method of re-feeding; when human milk cannot be given, alternative food sources must be sought. Soy or hydrolysed formula milks are good substitutes of human milk and also permit the children to grow up well, but they have not a good taste, therefore in some cases are refused by the infants. Furthermore it has been reported some cases of infant with intolerance to hydrolysed cow's formula or goat milks. Some findings conducted by Fantuz *et al.*, 2001; Salimei *et al.* 2004; Vincenzetti *et al.*, 2005a have demonstrated that donkey milk could substitute breast feeding in infants affected by severe Ig-E mediated milk allergies. In this case donkey milk is not only useful, but also safer than other types of milk. In fact donkey's milk composition in lipid and protein fraction, mineral content and lactose content is comparable to human milk. The new, current findings and analysis has come up with a solution for cow milky allergy since donkey milk substitutes and also more emphasis needs to be given to the protein fractions. It is considered to be a good alternative to infant formulas, since its nutrient composition, particularly the proteins, are very similar to those of human milk (Monti *et al.*, 2007).

2.5.1. Donkey milk total protein

Donkey's milk total protein content is the lowest (13 - 28 g/l); therefore it does not produce an excessive renal load of solute compared with that of bovine milk that contain 32 - 34 g/l (Salimei *et al.*, 2004) but it is very close to the values for human (9 - 15 g/l) and mare's milk (Doreau and

Boulot, 1989). In goat milk the total protein content is closest to that of bovine milk. The ratio caseins/whey proteins depend on species and it is high in rapidly growing mammals (lambs and calves). According to Saliemei and colleagues (2004), in donkey's milk the total casein content is about 8.7 g/l, which is very close to the value found in the human milk. Alpha s_1 - and B-caseins of caseins are identified. K- and Y-caseins can't be found probably due to low amount. The proteins that remain soluble in milk serum after precipitation of caseins at pH 4.6 at 30°C are whey protein. Similarly, its content in donkey milk is 1.8 mg/ml (Salimei *et al.*, 2004) and they are also variable at different stages of lactation periods.

About 35–50% of protein in donkey milk protein fraction is whey protein, while in cow's milk, it is only 20% (Herrouin *et al.*, 2000). A-lacto albumin and B-lacto globulin are of particular interest among whey proteins, this is because they are considered as the principal milk allergens in neonates and children (Carroccio *et al.*, 1999), but b-LG is absent in the human milk. Fantuz *et al.*, 2001, demonstrates that α -lactalbumin, b-lacto globulin and lysozyme are the most important whey proteins in equid milks. A-LA in donkey milk has two isoforms, called A and B, with different isoelectric points (Giuffrida, *et al.*, 1992) and it has a specific physiological function in the mammary gland, it interacts with the enzyme B-14-galactosyl transferase to form the lactose synthase complex and results in the modification of the substrate specificity of B-14-galactosyl transferase allowing the formation of lactose from glucose and UDP-glucose. The tightly bounded Ca^{++} that is contained in α -lacto albumin that influence the structure of protein does not affect lactose synthesis. According to studies carried out by Greppi and Roncada, 2005, the concentration of α -LA found in bovine milk is at an about 1.2-1.5 g/l (similar to the value in human milk (1.6g/l) and goat milk (1.2g/l) where as it is estimated 3.3 mg/ml in mare milk (Miranda *et al.* 2004; Greppi and Roncada, 2005). In the bovine milk, the concentration of α -LA is higher at the beginning of lactation and decreases near the end of lactation. This decrease is correlated to the decrease of the lactose concentration at the end of the lactation.

Donnet, 2000, discovered the existence of several variants of B-lacto globulin in donkey's milk which mainly exist as A or B variant. Accordingly there are three genetic variants of b-LG are found, these are: I-like b-LG with three amino acid substitutions, while the other two are similar to b-LG with two amino acid exchanges (Herrouin *et al.*, 2000). Structurally b-lacto globulin is

similar to retinol-binding protein and lipocalins. This may suggest that B-LG may have a role in the transport of fatty acids and vitamin A.

2.5.2. Nutritional sufficiency of donkey milk

Professionals in the field agreed and universally reached on consensus that breast milk is the perfect food for newborns with healthy mothers who have appropriate nutritional stores (Carroccio, 1999). Due to many reasons and health problems, in some cases babies cannot be breast-fed. So, it is necessary to find out what kind of milk and from which species or animal is the most suitable for babies. This is one of the important issues that this review of literature tried to address. One of the challenges this time around Cow's milk is allergy which is a common disease which causes sickness of infancy. Infant formula, an industrial milk product usually based on either cow or soy milk, strives to be an adequate artificial substitute for natural breast milk. However, since breast milk cannot be fully reproduced and sometimes babies can suffer from allergy when fed infant formula so that donkey milk can fill this gap (Carroccio *et al.*, 2000).

Researchers in the field believe that the positive results from the pediatrics trials are due to the particular composition of donkey milk protein fraction. According to Donnet, *et al.*, 2000, in addition to the traditional nutrients of the diet, bioactive molecules in functional foods, including milk, are clearly important in providing hormonal, antibacterial, probiotic or pharmacological support. In this regard, the properties of donkey milk could be due to its probiotic activity, high lactose content, (Coppola *et al.*, 2002) the presence of trophic and protective factors, immunoglobulin, peptides with antibacterial and hormonal activity, its excellent nutritional value and easily digestibility (Miranda *et al.*, 2004), in addition to its unique nitrogen composition, with a low casein-to-whey protein ratio makes it a good alternative to human milk in infant nutrition. Some of these protective factors, often characterized by inter specific activity, are also present in dairy cow's milk, but may not survive the technological processing used during the preparation of infant formulas. It is also important to consider the high lysozyme content of donkey milk in relation with its probable role in preserving the intestinal ecosystem from potentially harmful

microbes. The use in dermatology, which is well known since ancient Rome is also efficacious in smoothing skin and scalp inflammations.

Substances with bioactive properties are also in the category of lipids increasing importance is now attributed to the milk content of mono-di-and triglycerides, free fatty acids, phospholipids, glycolipids and steroids along with minor constituents, like carotenoids, vitamins and lipoproteins (Dugo *et al.*, 2005). Bearing in mind the fatty acids compositions of milk, most interest is actually addressed to polyunsaturated fatty acids, essential in immune-modulating activities, growth (Gill *et al.*, 2000) as well as in the development of the neonatal nervous system. In this regard, results of a study conducted by Doreau *et al.*, 2002, Shows the interesting free fatty acids profile of donkey milk, with a lower total saturated fatty acids content, an average higher level of total unsaturated fatty acids. Those experimental observations could support the positive contribution of donkey milk to an efficient cerebral growth and the development of a normal and complete immunitary system in the neonate (Carroccio *et al.*, 2000).

Overall, the good palatability of donkey milk and the very close chemical composition compared to human milk seem to encourage the use of this product in infant feeding especially in cases of multiple food hypersensitivity, lactose intolerance. Although the mechanism of this tolerance has not yet been fully clarified, it is reasonable to hypothesize that the reduced allergenic properties of donkey's milk can be related to structural differences of its protein components with respect to bovine milk. The high lactose content, the characteristic protein profile with a lower casein content (50 %) with respect to cow's milk (80 %) and the relevant percentage of essential amino acids make donkey milk a potential new dietetic food and a promising breast milk substitute.

2.5.3. Use of donkey milk as human milk substitutes

In any ratio and structure of milk constitutes, it has been found out that the composition of human milk is quite different from that of other mammalian milks. The protein content in human milk is closer to that of donkey and mare milk. Conversely, it is lower than in milk of ruminant dairy animals, cows, buffalo, camel, goat, sheep (El-Agamy *et al.*, 1997). The ratio of casein within total protein is lower in human milk, because whey proteins (soluble proteins) are higher than in

cow, buffalo, and sheep milk, whereas they are at similar level in donkey and mare's milk (El-Agamy *et al.*, 1997). This condition gives human milk the special property of forming a soft curd during digestion in the infants' gut. The softness of the curd is due to the lower ratio of soluble calcium. This condition may explain, why in many parts of the world mare and donkey milk are used as human milk substitutes for bottle fed infants (El-Agamy *et al.*, 1997). On the other hand, both cow and buffalo milk gives a hard curd, which of course is preferred in cheese making.

Prior to using for baby feeding, dilution of bovine milk with water must be practiced for safe nutrition, especially for very young babies. In contrast, human milk proteins are different in their composition and structure from those of the milk of other species. It is known, that the major whey proteins of bovine milk are β -lg with 55% of total whey proteins, alpha lactoalbumin with 20%, and blood serum albumin with 7% (Taylor, 1986). These proteins differ in their types and ratios between goat, sheep, cow, camel, human, buffalo, mare and donkey milks (El-Agamy *et al.*, 1997). Human milk is free of beta lacto globulin, one of the major allergens in cow milk, similar to camel milk, which also has no beta lactoglobulin (El-Agamy, E.I., 2006c). On the contrary, beta lactoglobulin is a major whey protein in cow, buffalo, sheep, goat, mare and donkey milk (El-Agamy *et al.*, 1997). Caseins in the milk of these species differ in fraction number and their electrophoretic behavior on polyacrylamide gel, amino acid composition and their peptide mappings (El-Agamy *et al.*, 1997).

Donkey milk has several advantages over cow's milk for human consumption. Recently, cow milk protein allergy has drawn the attention of researchers over the world. It is an altered or abnormal reaction. Such a reaction may occur when there is contact between a foreign protein "an allergen" and body tissues that are sensitive to it. The allergy may reach the tissues by direct contact with the skin mucous membranes or through the blood stream after absorption. Allergic reactions have been classified into two types: immediate reaction type and the delayed reaction type. Cow milk allergy is clinically an abnormal immunological reaction to cow milk proteins, which may be due to the interaction between one or more milk proteins and one or more immune mechanisms, and resulting in immediate IgE-mediated reactions. On the other side, reactions not involving the immune system are defined as cow milk protein intolerance. CMA occurs in some infants after ingestion of an amount of cow milk (Goldman *et al.*, 1963).

Hypersensitivity to cow milk proteins is commonly one of the main food allergies and affects mostly infants and also adults in infrequent cases. Cow milk protein allergy may be very severe in some cases. Different clinical symptoms of milk allergy have been established. The diagnosis of milk allergy differs widely due to the multiplicity and degrees of symptoms, and can be achieved by skin or blood tests. Cow milk contains more than 20 proteins that can cause allergic reactions. Casein fractions and b-lacto globulin are the most common cow milk allergens (Aalberse, 2001).

With the introduction of new and different procedures, some possibilities are adapted in reducing the allergen-city of milk proteins. Different attempts have been made and various technological processes have been applied in order to have better use of cow milk in infant formulae. These are heat treatment, infant formula, enzymatic treatment, extensively hydrolysed formula, amino acid-based formula and soy formula. However, this is a bit costly and requires various procedures, so that using, camel, mare or even soy milk and donkey milk as alternatives in cases of cow milk allergy. Milk from these spices can be considered hypoallergenic. Allergies to milk proteins of non-bovine mammals have also been documented due to cross-reactivity between cow milk proteins and their counterpart in other species, and even between goat and sheep caseins. Genetic polymorphisms of milk proteins play an important role in eliciting different degrees of allergic reactions. However, therapeutic benefits vary with the degree of severity of the allergy (El-Agamy, 2006c).

Chapter - 3

MATERIAL AND METHODS

3.1. Study area and study animals

Adea woreda is found in Eastern Shewa zone of Oromia regional state in Ethiopia. It has 27 rural kebeles and each has 2-4 villages. Big towns like Bushoftu are found in this woreda. For this specific study, three rural kebeles namely Garaboru, Babogaya and Hiddi were selected. The total human population in these kebeles is about 17600. With respect to livestock population, these kebeles have more (Table 1) Cattle, Sheep, Goat and Donkey population than other remaining kebeles in the woreda. As indicated in the Table 2, there are more than 9845 donkey population in the three kebeles in which 3920, 2144 and 1697 are found in Gara boru Babogaya and Hiddi respectively (Adea woreda agricultural and rural development office, 2008). The report from Adea woreda agricultural and rural development office (2008) indicates that majority of the donkeys are male donkeys. The livelihood of the population of the woreda is mainly based on crop production and livestock as the 2nd means of livelihood (priority area). The major crops produced in this area include 'Teff,' wheat and highland pulses such as pea, feba-bean and chick pea. Donkey is one of the means of transporting the crop from the field to the home and market areas. This implies that donkey has an importance for the livelihood of the peasant in this area.

Table 2. Human, Livestock and donkey population in Garaboru. Babgaya and Hiddi

Area	Human population			Livestock population					
	M	F	Total	Cattle	Sheep	Goat	Donkey	Horse	Mule
Garaaboru	2587	2152	4739	6688	739	220	3920	2	3
Babogaya	3117	2958	6075	3768	837	715	2144	117	23
Hiddi	3705	3081	6786	6921	987	418	1697	152	37

Source: Report from Adea woreda agricultural and rural development office (2008).

There are four types of donkeys in Ethiopia namely Abyssinia, Ogaden, Jimma and Senar donkeys. Among these types, Abyssinia donkeys were selected for this research purposively. To this end, Adea woreda was selected since large population of Abyssinian donkeys are found in this area. Thirty donkeys, (parity from one to five), selected from the above mentioned areas were used in this study. A preliminary examination was conducted by using 0.1 M sodium hydroxide solution to ensure that the experimental donkeys are free from mastitis.

3.2. Sample collection

Samples were collected from 30 Jennies which are found in different kebeles of Adea woreda. From each jenny, approximately 500 ml of the milk was taken to represent content of the whole population. The collection and transportation of the milk samples were synchronized. The milk was collected on the same day and transported to both laboratories namely micro-biological and food technology laboratories. The reliability of data regarding the content of any component depends highly on correct sampling, transportation, storage and adequate analytical methods in order to minimize the sources of error. Glasses used for the milk samples were acid washed to minimize impurities consisting of trace elements especially for mineral analysis. In addition pH also was checked at the time of arrival at food Engineering laboratory.

Samples were milked manually from apparently healthy Jennies and sampling was continued until the desired sample size was obtained. Samples were collected through direct milking into a sterile test tube/bottle from washed and unwashed udder, for nutritional and microbiological analysis respectively, and transported to laboratories within 3-4 hours using an ice box to maintain at 4 °C.

3.2.1. Sample preparation

The samples were then thoroughly mixed, sub-sampled and samples for the analysis of ascorbic acid and vitamin E were stored in brown bottle. Consequently, samples were transferred to a deep freezer and stored at -20 °C until nutritional analysis was conducted. Microbiological and physical analyses were carried out immediately at arrival in the Drug Research Department of Ethiopian Health and Nutrition Institution, microbiological and Food Engineering laboratories, respectively.

3.4. Instrument and methodology

The parameters and the analytical methods are given in Table 3 below. The parameters analyzed were the main components of milk such as total protein, total fat, lactose, as well as other components of significance from nutritional and microbiological point of view. Components were analyzed from all 24 Jennies (24 samples in total). The methods of analyses followed were that of the Association of Official Analytical Chemists (AOAC) and reference methods of the International Dairy Federation (IDF) and other published methods.

Table 3. Analysis methods used for the quantification of components of donkey milk

Proximate analysis	Methods of analysis
Fat	Gerber method
Protein	Kjeldahl methods
Lactose	Lactoscan method
Vitamin C	Spectrophotometric method
Calcium	Standard method of AOAC (1998)
Magnesium	Standard method of AOAC (1998)
Zinc	Standard method of AOAC (1998)
Iron	Standard method of AOAC (1998)
Phosphors	Spectrophotometric method (1998)
PH	pH meter
Total solid	IDF-ISO-AOAC method (1999)
specific gravity	pycnometric method
Acidity	Titrimetric method (AOAC, 1947)
Freezing point	Lactoscan method
Isolation and Identification of microbes	International standard ISO 8261.

3.4.1. Physical analysis

PH was determined by using a digital pH meter (pH test 10 DOUBLE JUNCTION Eutech instrument), and density was determined at 15°C by pycnometric method (AOAC, 1999). Total solids were determined gravimetrically after the milk was dried in a forced-convection oven at 105°C until a constant weight was achieved according to IDF-ISO-AOAC method (AOAC,

1999), while acidity was determined by titrimetric method (AOAC, 1947). Acidity is reported as % lactic acid by weight in which 1 ml of 0.1M NaOH = 0.0090 g lactic acid.

3.2.2.2. Proximate analysis

Fat content was determined by using Gerger-method. Prior to analysis, each sample was warmed to 38°C in water bath to disperse the cream and reincorporate any cream adhering to container or stopper and mixed until homogenized. Then the warmed test samples were cooled to 20°C. Following this, 10.77 ml of pre-treated samples were poured into butyrometer containing 10 ml of sulfuric acid. One ml of amylalcohol was added into the butyrometre and shaken until the content is thoroughly mixed; i.e, until no white particle of milk could be seen by inverting the butyrometer during the process. The butyrometers were placed into the Gerber which maintain a temperature of 65°C for 5 minutes while centrifuging at speed of 1100 r.p.m. The fat percentage was recorded by observing the fat column.

Nitrogen content of donkey milk was determined by the Kjeldahl method and a nitrogen conversion factor of 6.38 was used to calculate protein contents of donkey milk samples. Donkey milk was digested in H₂SO₄, using CuSO₄·5H₂O as catalyst with K₂SO₄, as boiling point elevator, to release nitrogen from protein and retain nitrogen as ammonium salt. Concentrated NaOH was added to release NH₃, which is collected in H₃BO₃ solution and titrated.

Volume of HCl was recorded and the results were calculated by using the following formula:

$$\text{Nitrogen, \%} = [1.4007 \times (V_s - V_b) \times M] / W$$

Where V_s and V_b = mL HCl titrant used for test portion and blank, respectively; M = molarity of HCl solution; and W = test portion weight, g. Percent nitrogen was multiplied by factor 6.38, to calculate percent "protein" on a total nitrogen basis.

The milk constituent's lactose and physical characteristics such as freezing point of the milk were determined by milk analyzer Lactoscan 90 LCD (Aple Industries services–La Roche Sur Foron, France) based on manufacturer's instructions. Milk samples were mixed gently to avoid any air enclosure in the milk. Then 25 ml samples were poured in to the sample-holder with the

analyzer one at a time and when the analyzer sucks the milk, The measurements, was carried out after the starting button was activated. Finally the digital indicator display shows the specified results.

Vitamin C content of donkey milk was determined using spectrophotometric method. Milk samples were centrifuged at 3000 rpm for 20 minutes. The fat component of milk separates from the fluid at the top, and the cells settle at the bottom. From the clear mid-zone, 0.3 ml of milk was taken and 1.2 ml trichloroacetic acid was added and mixed well. The mixture was then centrifuged at 3000 rpm for 10 minutes. 0.9 ml of the supernatant was taken and 0.4 ml of 2, 4 dinitrophenyl hydrazine thiourea copper sulfate) solution was added. It was then covered with aluminum foil and incubated at 60°C for 60 minutes in a water bath. Then samples were chilled in ice-cold water immediately and 1.6 ml of 65% sulfuric acid was added gradually. Then the treated sample was stored at room temperature for 30 minutes. Absorbance was measured against a reagent blank at 520 nm by a spectrophotometer (UV-v3, Thermo Electrocorporation). Every sample was analyzed twice to obtain duplicate readings (Luthfor *et al.*, 2004)

In preparation of standard, 1000 mg/dL (w/v) ascorbic acid stock standard was made by dissolving 1g of L-ascorbic acid in 30 ml of distilled water in a 100-mL volumetric flask and mixed well and dilute to volume. From this 5 ml was taken and added into a 200-ml volumetric flask and diluted to volume with distilled water to form intermediate standard. Finally in a series of 50-ml volumetric flasks (Elaine, *et al.*,1976-1980) , working standard solution was prepared (Table 4 and Figure 1).

Table 4. Working Vitamin C standard preparation

Standards	ml Intermediate Standard	Actual Cone. [mg/dL] Standard Solution
1	0	0
2	4	2
3	8	4
4	12	6
5	16	8
6	20	10

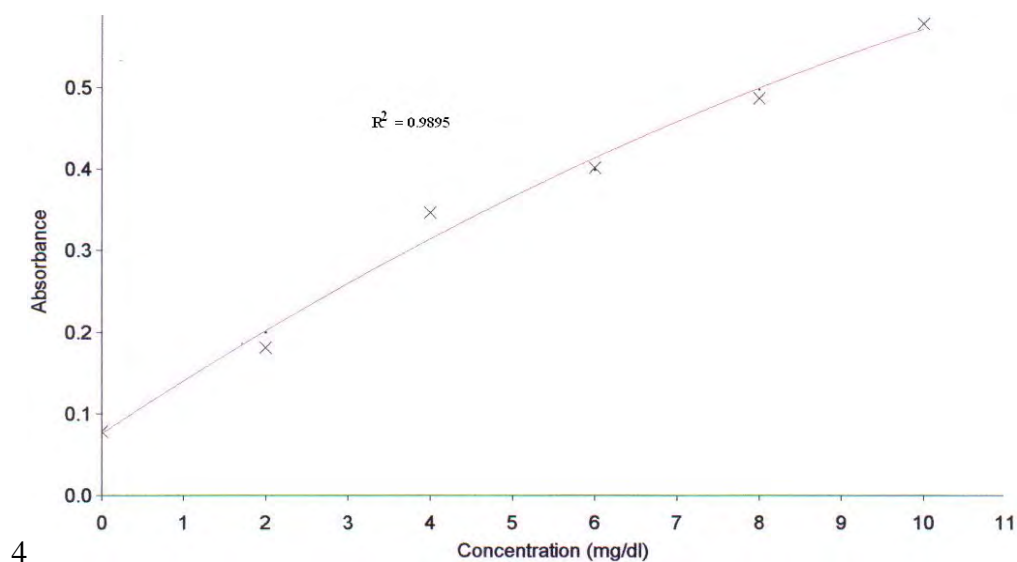


Figure 1: Standard curve for ascorbic acid determination

34.3. Mineral analysis

The analytical determination of the macro elements Ca, Mg, and the micro element Fe and Zn were performed using an Atomic Absorption Spectrometer (Varian, Spectra 220). After removal of organic matter by wet digestion, liquate was diluted with water according to standard Association of Official Analytical Chemistry method (1998). On the other hand, phosphorous was determined by spectrophotometer method from wet digestion test portion by complexing with ammonium molybdovanadate reagent. Twenty milliliter of molybovanadate and 20 ml of

pre-prepared test solution was added to 100ml of volumetric flask and diluted to the mark with distilled water. After 10 minutes, phosphorous was determined in the form of phosphate at 400 °C.

The inorganic acids (concentrated HNO₃ and H₂SO₄) used were all of analytical grade and a materials and glasses were thoroughly cleaned with HNO₃ to 10% v/v. Samples were prepared in a very clean place under clean conditions in Ethiopian standard and quality authority laboratory. The methodology used, consisted of introducing 5.0 mL sample of milk which was weighed and placed in Kjeldahl flask (capacity of 100 mL). Following, 10.0 mL of concentrated H₂SO₄ and 5.0 mL of concentrated HNO₃ were added. After that, it was cautiously heated until the initial vigorous reaction has subsided. Heating was continued until most of the nitrous fumes are removed. Then HNO₃ was added drop wise until all organic matter is destroyed and heated until white fumes of sulfuric acid was evolved and solution become colorless. Blank was prepared using the same amounts of reagents as sample. Solutions obtained from wet digestion was transferred from the flask to a graduated volumetric flask and filled up to mark with water and mixed thoroughly. Finally, measurements of the elements content were carried out following the instruction given for the relevant metal.

The apparatus was set according to the instructions of the instrument. The standard and the reagent blank solution were measured and calibration curve was prepared by plotting the absorption values against the metal concentration in mg/L. This task was performed automatically by atomic absorption by providing only one solution of known concentration of each element for Ca, Mg, Fe and Zn, unlike phosphorous which involves preparation of series of working standard solution of 0.0, 5.0, 8, 10 and 15 (Table 5 and Figure) for plotting calibration curve on spectrophotometer. Then P was determined by using the following formula:

$$P = 100 * V_2 * p / V_1 / W$$

Where: W= Weight of samples

V₁= Volume of dilution

V₂= Volume molybovanadate solution

p = Concentration [mg/l] phosphate ion

P = Phosphorus content (mg/kg)

Table 5. Working standard for Phosphorus determination

Standards	Actual Con. [mg/l] Standard Solution
1	0
2	5
3	8
4	10
5	15

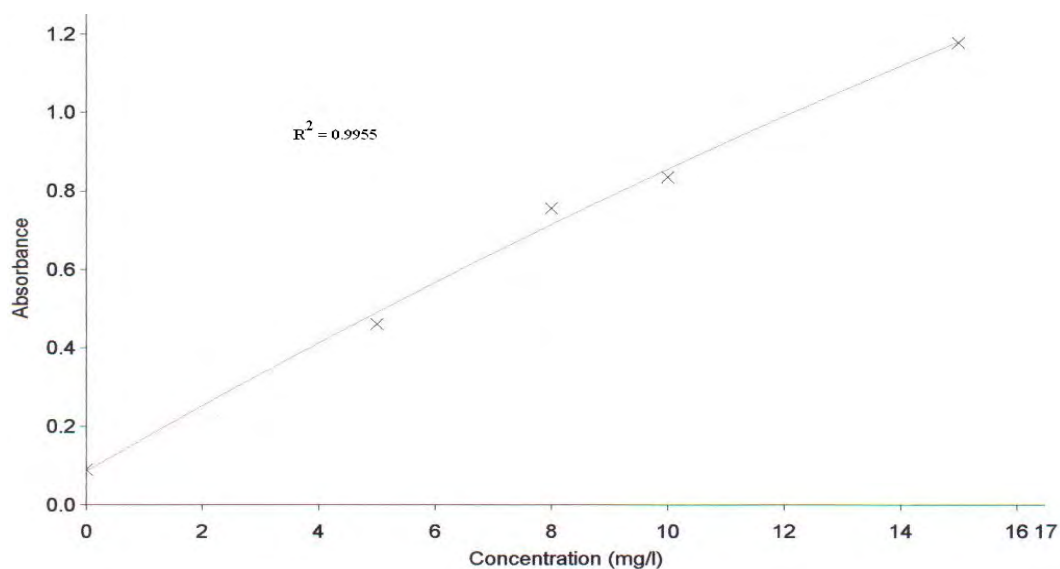


Figure 2: Standard curve for phosphorus determination

The concentration [mg/l] of specific metal (Ca, Mg, Fe and Zn) that correspond to the absorption values of the samples and blank were read from the graph and calculated.

$$\text{Metal content (mg/kg)} = [(a-b) \times V]/$$

Where: W= Weight (g) of samples

V= Volume (V) of dilution

a = Concentration ($\mu\text{g/ml}$) of sample solution

b = Concentration ($\mu\text{g/ml}$) of blank solution

3.4.4. Microbiological analysis

This study was conducted in Addis Ababa at Ethiopian Health and Nutrition Research Institute (EHNRI) at the department of Drug research and Institute of Biodiversity. A minimum of 45 to 50 isolates were isolated from each sample on different media at different temperature of incubation, most of which later on are reclassified in to few groups on preliminary biochemical test. These samples were again re-grouped into three main groups each of which has its own 4 sub-groups. First batch of samples are raw donkey milk samples collected from individual farmer's homes located at about 10 km on the southern direction of Adea town from farmer association specifically known as Gara Boru. These samples were used to monitor changes in microbial spectrum and population kinetic of raw as well as fermented (further incubated) donkey milk samples. Second batch of samples are raw donkey milk samples collected from individual farmers homes located at about 5 km on the southern direction of Adea town from Babogaya area as mentioned for Firs Batch Sample. Third batch of samples are raw donkey milk samples collected from individual farmers homes located at about 20 km on the southern east of Adea town called Hiddi area for the same analysis as mentioned under first batch and second batch of samples. The samples were immediately transported to the laboratory for zero-hour sampling. In each case samples were collected, in pre-sterilized bottles with a holding capacity of 30 ml. Then fermentation was initiated in these bottles with the left over samples after portions of samples are taken for zero hours sampling.

Fermentation process was allowed to proceed for about 18 days at room temperature as it was not easy for donkey milk to ferment within this time of incubation unlike cow milk. Aliquot (10 ml) of the milk sample was taken for each bacteriological analysis after homogenization of each sample was done under aseptic conditions. Both general as well as selective media were used for isolation of various groups of microbial colonies. PCA (plat count Agar), PDA (potato Dextrose Agar), M17 (M17- Agar), MRS (Mann Rogosa Sharpe Agar), and MacConkey were used to cultivate and isolate Total Aerobic bacteria (TAB), Fungi (Yeast and Mould), G.P (gram positive), and gram negative groups respectively. To grow and isolate each expected organism, pour plate method was used for all media listed above; i.e., to each plate 15 to 20 ml of molten agar at 45⁰c to 50⁰c was added. Then 1 ml of inoculums from each dilution is required into each

Petri-dish. The medium in each plate and the inoculums was mixed by shaking and circular movements lasting 8 sec to 1 minute. All plates were allowed to set until solidified.

Each plate was then inverted and incubated at appropriate temperature. All the plates are labeled fully (i.e. the label contained at least name of the media, dilution factor, temperature of incubation, code of the sample, time of the experiment). All counts were determined by serially diluting the milk samples 10 fold. In this case, after the milk samples were homogenized, about 10 ml of the sample was added to 90 ml of buffered peptone water to prepare the first 10 fold dilution in each case then this was further diluted up to 10^6 using again the same buffered peptone water. One ml was inoculated in duplicate from each dilution on each medium to obtain countable colonies in each plate.

Incubation was carried out at 30°C for M17 and MRS plates (gram positive) and at 37°C for PCA (Total Aerobic Bacteria) and MacConkey (gram negative group) plates, While it was at 25°C for PDA (Yeast and Mould) plates. The incubation periods were 24 to 72 hrs for all. But sometimes when there is no good growth the incubation period was extended up to over 1 week. Plates having between 30 to 100 colonies were counted and colony forming units per milli litter (cfu/ml) of test samples were calculated.

The major of the preliminary biochemical tests were carried to group them at least into genus and group level for isolates of each sample. Some of the main preliminary biochemical tastes are like, Catalase test, Oxidase test, morphology (using Phase Contrast Microscopy technique). Cultural Characters and morphology were the primary methods used as the first step, in identification & characterization of the colonies for further biochemical tests. Purification of each colony for further biochemical tests to classify each colony (isolate) to at least genes level was done on PCA plates i.e. each isolate was re-streaked on solidified PCA plate to be checked for purity before running any biochemical test. Finally each pure organism was transferred in to slant in their respective media and stored at 4°C .

Identification and characterization of gram positive microorganism

Identification and characterizing of microorganisms were performed following procedure given by OmniLog Identification manual. Before the identification, a pure culture was isolated on OmniLog Bug Agar and Gram Stain was done. The inoculums cell density was prepared using turbidometer which was set according to the Gram staining classification. The prepared inoculums were introduced in a labeled microplate and were inoculated in a strictly aseptic condition. The microplates were placed in OmniLog Plus ID Chambers after necessary data were loaded to ID system. The OmniLog ID provided reading of results and hard copy prints t after an ID was completed (Biolog inc., 2006)

3.5. Statistical Analysis

Data were analyzed by SPSS program (Statistical Package for Social Science, version 15.00. 2007). This test combines ANOVA with comparison of differences between means of the location.

Chapter – 4

RESULTS AND DISCUSSION

4.1. Physicochemical analysis

Physical characteristics of milk (such as pH, density, titratable acidity, total solids) are important parameters in studying its physicochemical aspects. Table 6 shows the various physical parameters of the Abyssinian donkey milk samples.

Table 6: physical properties of Abyssinian donkey milk samples collected from the study sites

Parameter	N	Mean \pm SD	CV	Minimum	Maximum
pH	24	7.20 \pm 0.12	0.016	7.0	7.40
Density [g/cm³]	24	1.026 \pm 0.006	0.0058	1.01	1.04
Acidity [%]	24	0.14 \pm 0.15	1.07	0.04	0.53
TSS [%]	24	8.80 \pm 0.98	0.11	6.56	12.16
F point [⁰C]	24	-0.45 \pm 0.065	-0.144	-0.56	-0.27

PH is the parameter that determines the sample acidity and alkalinity. The mean level of pH of donkey milk was found to be 7.2 \pm 0.12. The coefficient of variation in the same table (0.016) shows that the pH level did not vary among individual donkeys. Compared with this statistic, Guo and colleagues (2007) found out a pH level of 7.18 \pm 0.13 for donkey milk and 6.89 \pm 0.08, for human milk (Table 1). Salimei *et al.*, (2004) indicated that the pH level for donkey milk was in the range of 7.14 to 7.22. Comparison of the pH value of the present study with the literature value for donkey milk suggested that there is no statistically significant difference between the two ($t_{92} = 0.66$, $P > 0.01$, Table 9) while the difference is significant with the human milk ($t_{42} = 8.61$, $P < 0.01$, Table 9). This suggests that the pH value may not be influenced by possible breed variation for donkeys. The average pH value (7.2) of donkey milk was higher than cow milk pH ranges from 6.3 to 6.8 (Van den Berg, 2006) which is slightly acidic.

Milk density is another important parameter. It is influenced by factors like inclusion of air during delivery, adulteration of water and skimming. So, the density measurement of milk is used for quality control. Density of donkey milk in this investigation has the mean value of 1.026 \pm 0.006. This value is similar with literature (Guo, *et al.*, 20007) for both donkey (1.03 \pm 0.02,) and human milk 1.027 \pm 0.011 (Table 1) because, the mean comparison of the present donkey milk

with literature donkey milk data ($t_{172} = -0.968$, $P > 0.01$, Table 9) and also with human milk ($t_{42} = 1.30$, $P > 0.01$) presented on Table 9 shows that there is in significant difference between the means.

As an indicator of milk quality, milk acidity is used to measure and monitor such processes as making cheese and yogurt. Accordingly, due to the buffering capacity of protein and milk salts, fresh cow milk exhibits an initial acidity of 0.14 to 0.16 (O'Connor, 1995) when titrated with 0.1 NaOH N. In this study, the acidity value obtained for donkey milk was in the range of 0.53 to 0.14 which is lower than cow milk acidity. When Acidity of donkey milk investigated in this study was compared with human milk (0.072 ± 0.011 , Table 1) Elisabetta and colleague (2009), no statistically significant difference was found between the two means ($t_{172} = 1.98$, $P > 0.01$, Table 9).

Finally, the dry matter observed in current donkey milk study (average of 8.8 ± 0.98 %) was also compared with the one in the literature (Chiavari, 2005) which reported a mean average of (8.91 ± 0.31 , for donkey and 10.71 ± 0.11 (Table 1) for human milk. The mean test shows that there is a significant difference between the two mean ($t_{42} = 8.47$, $P < 0.01$), while the dry matter content of the current donkey milk is consistent with the donkey milk content in literature ($t_{172} = -1.07$, $P > 0.01$ Table 9). The means of freezing point of donkey milk samples studied was -0.45 °C. The result obtained in this study is greater than freezing point for cow milk as investigated by Samia *et al.*, (2009).

4.2. Proximate analysis

Protein, Fat and Lactose are among the basic group of milk component. The Abyssinian donkey milk samples were also subjected to the determination of the amount of total protein, fat, lactose, vitamin C as presented on Table 7. Minimum and maximum values, overall means of gross composition, standard deviation, and coefficients of variation are reported in this Table.

Table 7. Composition of Abyssinian donkey milk samples collected from Garaboru, Babogaya and Hiddi area

Item	N	Mean (\pm SD)	CV	Minimum	Maximum
Lactose [%]	24	6.29 \pm 0.45	0.07	5.39	7.01
Fat [%]	24	0.36 \pm 0.11	0.30	0.23	0.67
Protein [%]	24	1.98 \pm 0.40	0.17	1.43	2.89
Vitamin C [mg/dl]	24	3.15 \pm 1.18	0.37	1.25	6.23

The observed average protein content of the present donkey milk (1.98) was also compared with the one in literature for donkeys (1.56 \pm 0.14, Table 1) and humans (1.94 \pm 0.14, Table 1). Results revealed that it was consistent with Elisabetta *et al.*, (2009) data for human milk ($t_{42}=0.048$, $P>0.01$, Table 9) but significantly different from its own species ($t_{42}=8.38$, $P<0.01$, Table 9).

Fat is the most variable component in milk both in its concentration and chemical composition, despite species differences. Milk fat is the major source of lipid used by the mammalian newborn for accumulating body adipose tissue. The data in this study revealed that the average fat content of donkey's milk investigated in this study indicated in Table 7 was 0.36% \pm 0.11 and similar with the values reported by Salimei *et al.*, (2004) for donkey (0.38% \pm 0.12, $t_{92} = 0.36$, $P>0.01$,) but lower than Elisabetta and colleague' (2009) reported for human milk (2.1% \pm 0.15, $t_{42} = 43.25$, $P<0.01$, Table 9).

Lactose is a component of milk considered. It is unique to milk (i.e. does not exist anywhere else in nature) and it plays a major role in milk synthesis. Because of the close relationship between lactose synthesis and the amount of water drawn into milk, lactose is the least variable component of milk. Lactose is the major milk carbohydrate in most species. Some species have very little lactose in milk (e.g. bear and kangaroo), while others (e.g. donkey, human) have high as 7% lactose in their milk. Lactose is important in the manufacturing of many fermented dairy products such as yogurt (O'Connor, 1995). Considering our present data summarized in Table 7, the high lactose content determined in this study (mean of 6.29 \pm 0.45) was consistent with Guo *et al.*, (2007) reported data for donkeys (mean 6.33 \pm 0.17, $t_{92}=-0.62$, $P>0.01$), and humans (mean

6.45± 0.08, $t_{42}=-0.7$ P>0.01). Finally, the mean vitamin C content in the present donkeys (3.15 ± 1.18) was compared with human milk in literature (3.06 ±0 .67) (Luthfor *et al.*, 2004). And test results on Table 9 yielded that there is no significant difference between the two.

4.3. Mineral Analysis

Table 8 summarizes the result analysis for Ca, Mg, Zn, Fe, and P contents in Abyssinian donkey milk samples collected from the study sites

Table 8. Mean (\pm SD) of mineral composition of Abyssinian donkey milk samples collected from Garaboru, Babogaya and Hiddi are

Milk Contents	N	Mean	CV	Minimum	Maximum
Calcium [mg/kg]	24	737.46 ± 309.85	0.42	435.73	1895.32
Magnesium [mg/kg]	24	58.71 ± 22.40	0.38	30.11	137.13
Iron [mg/kg]	24	24.06 ±28.05	1.17	3.54	94.73
Zinc [mg/kg]	24	5.56 ± 3.38	0.61	1.68	14.84
Phosphors [mg/kg]	21	314.99 ± 151.69	0.48	87.25	0.63

Mean Ca content of donkey's milk was 737.46 ± 309.85 mg/kg, almost closer to Salimei *et al.*, (2004) reported value for donkey milk (mean, 677 ± 282.85 mg/kg, $t_{46} = 0.69$, P>0.1). But one fold higher when compared to a previous study by Elisabetta *et al.*, (2009) for donkey milk (334.61 and human milk (340.00 mg/kg, $t_{42} = 5.87$, P<0.01). There is significance difference between what was investigated by this study and previous report by Elisabetta *et al.*, (2009). Moreover, the observed Ca content was higher than that reported for cow's milk (680 ± 79.8 mg/k) or other milk from ruminants such as goat (646 ±76.6 mg/kg) Elisabetta *et al.*, (2009).

Mean Mg content of donkey's milk (58.71± 22.4 mg/kg) is 2-3 times lower than from the one reported (Mohammad, *et al.*, 2008) for goat (139.15.7 mg/kg) and bovine milk (205.22.1 mg/kg) but almost similar with that reported in the literature for donkey milk (58. 46 ± 8.43 mg/ kg) by Elisabetta *et al.*, (2009) ($t_{46}= 0.14$, P>0.01). The Mg content observed in the present study was, however, significantly higher than what was previously observed in human milk (30± 0.35

mg/kg) by Elisabetta and *et al.*, (2009) ($t_{42}=79.31$, $P<0.01$). The same can be said with the Mg content in human milk previously reported to range from 15 to 64 mg/L with a median value of 31 mg/L (Dorea, 2000). Mean Zn content of donkey's milk obtained in this study (5.56 ± 3.3 mg/kg) was significantly different from what was observed in human milk (2.1 mg/kg, $t_{42}= 4.2$, $P>0.01$) and donkeys' milk (1.22 ± 0.52 mg/kg, $t_{46}= 6.1$, $P>.01$). It was demonstrated that Zn in human milk is more efficiently utilized compared to bovine milk because of the high casein content may be the same is true for donkey milk due to close similarity of chemical composition.

Table 9. Test of differences between contents of Abyssinian donkey milk in the present study and literature values for human milk and donkey milk

Parameters	Abyssinian and literature donkey milk		Abyssinian and literature human milk	
	df	t _{calculated}	df	t _{calculated}
pH	92	0.66	42	8.61
Density	172	-0.968	42	1.30
Acidity	-	-	42	1.98
TSS	172	-1.07	42	8.47
Fat	92	0.36	42	43.25
Protein	92	8.38	42	0.048
Lactose	92	-0.62	42	-1.54
Vitamin C	-	-	48	0.33
Calcium	46	0.69	42	5.87
Magnesium	46	0.14	42	79.31
Iron	46	3.92*	42	3.69*
Zinc	46	6.10	42	4.20
Phosphorus	-	-	42	4.46

* All values are significant at $p<0.001$

The mean Fe content of donkey's milk (24.06 ± 28.05 mg /kg) was much higher than those observed by Elisabetta and *et al.*, (2009) for human (0.4 ± 0.0 mg/kg, $t_{42}=3.69$, $P<0.01$) and donkey milk (1.15 ± 0.51 mg/kg, $t_{46}= 3.92$, $P<0.01$). Fe content of donkey' milk showed a high variability, ranging from 3.54 to 94.74 mg/kg. In the present study, the mean content of P in donkeys' milk (314.99 ± 151.7 mg/kg) was higher than human milk (160 ± 0.85 mg/kg, $t_{42}= 4.46$, $P<.01$) (Elisabetta *et al.*, 2009).

4.4. Factors affecting donkey milk composition

Attempts were also made to determine if milk composition of the sampled donkeys could differ location-wise, and along with the lactation period. One-way ANOVA was conducted to test location effect (Table 10).

Table 10: Differences in physicochemical parameters and composition of Abyssinian donkey milk contents among the three study sites

Parameters	Mean Square	F	P-value
pH	0.106	16.440	0.000
Density	0.000	8.555	0.002
Acidity	0.010	0.444	0.647
TSS	0.485	0.482	0.624
Freezing point	0.006	1.493	0.248
Protein	0.97	0.907	0.576
Fat	0.024	2.179	0.138
Lactose	0.196	1.081	0.357
Vitamin C	0.481	0.324	0.727
Calcium	61509.011	0.619	0.548
Magnesium	969.364	2.19	0.145
Iron	2777.912	4.813	0.020
Zinc	42.524	4.726	0.020
Phosphorus	29023.820	1.299	0.297

As indicated in Table 10, it is only pH ($F_{2, 21} = 16.44$, $P < 0.000$) and density ($F_{2, 21} = 8.56$, $P < 0.002$) of the sampled donkey milk compositions that were found to significantly differ location-wise. The absence of difference is probably due to their similarity in their feeding system which is based on grazing and similarity in agro ecological condition.

4.5. Microbiological analysis

Table 11. Mean value of microbial count for TAC, GN bacteria, G.P positive, Yeast and mold in log CFU/g of donkey milk Samples collected from the three sampling sites

Location	Time of incubation	Expected microbial group				
		TAC [log cfu/ml]	Gram negative [log cfu/ml]	Gram positive [log cfu/ml]	Yeast [log cfu/ml]	Mold [log cfu/ml]
GaraBoru	0 hr	5.66	5.40	2.32	1.72	2.45
	18 days	6.32	4.81	3.38	4.69	1.40
BaboGaya	0 hr	4.62	5.01	3.08	1.18	1.25
	18 days	6.00	4.48	6.07	1.00	1.14
Hiddi	0 hr	3.99	3.11	3.15	2.72	2.30
	18 days	6.2	2.77	6.74	1.48	1.00
Mean total	0 hr	4.75	4.5	2.85	1.87	2.00
	18 days	6.17	4.02	4.75	2.39	1.18

The mean average of three sampling site initial donkey milk contained of mean 4.75 log cfu/ml of total count bacteria (TAC), gram negative, 4.5 cfu/ml, Gram positive 2.85 cfu/ml, 1.87 log cfu/ml of yeast and 2.00 log cfu/ml of mold (Table 11). At room temperature, TAC and gram positive bacteria increased progressively during storage for 18 days to the value of 6.17 and 4.75 log cfu/ml. While gram negative, yeast and mold reached the counts of 4.02 log cfu/ml, 2.39 log cfu/ml and 1.18 log cfu/ml respectively, under the same conditions. Initial raw donkey milk contained a low microbial content with a mean total count (TAC) of 4.75 log cfu/ml, while the loads of microbe of bovine milk were approximately 7 cfu/ml, (Chye, Abdullah, & Ayob, 2004; Morgan et al., 2003). The reason for the low microbial counts in donkey milk is likely due to the natural antimicrobial substances present in it.

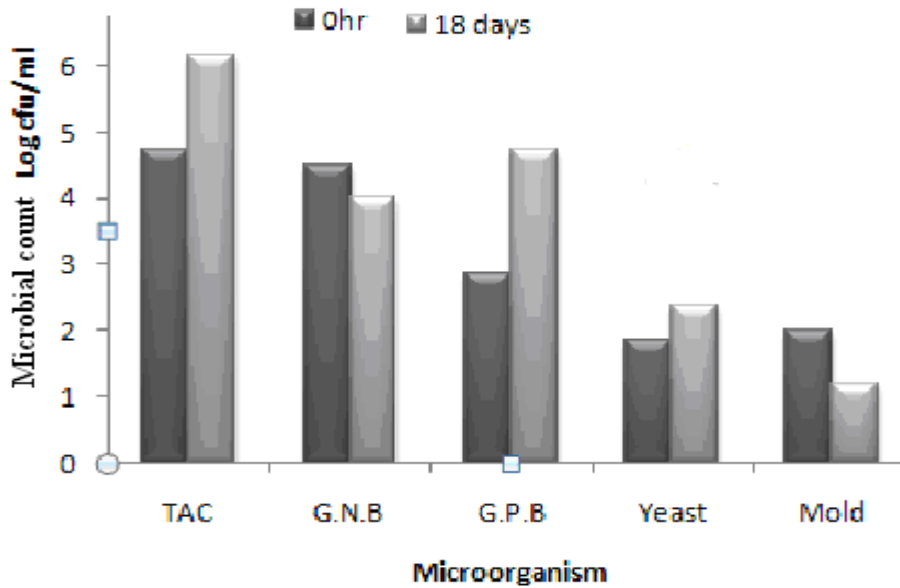


Figure 3. Change in microbial flora of Abyssinian donkey milk samples during storage for 18 days

During microbiological investigation Gram negative, Gram positive, aerobic bacteria, yeasts and moulds were isolated (Table 11). The isolates were initially differentiated on the basis of their cultural and morphological studies after which they were subjected to Catalase and Oxidase tests. A total of 150 colonies morphologically observed. Morphological identification of the isolates led to species identification of those gram positive bacterial isolate. From this 20 bacterial isolates suspected as lactic acid bacteria were used for further species identification by OmniLog Puls Identification System at the Microbial laboratory of the Institute of Biodiversity Conservation (Table 12).

Table 12. Bacterial strains isolated from Abyssinia donkey milk that were suspected as lactic acid

Strain Code	Microbial flora count [cfu/ML]		Identification	
	0 hrs	18 days	Morphological	OmniLog Plus System ID
DM-11	3x10 ²	2x10 ³	Cocci (mostly diplo cocci)	-
DM-29	8x10 ¹	-	Rod (short)	<i>Clavibacter agropyri</i> (corynebacterium)
DM-31	6.2x10 ²	2x10 ³	Rod (short)	-
DM-33	2x10 ²	1x10 ³	Rod (very short)	-
DM-34	1x10 ²	4x10 ³	Rod (very short)	-
DM-69	3x10 ²	4x10 ⁵	Cocci (mostly diplo cocci)	-
DM-77	3x10 ³	3.52x10 ⁶	Cocci (mostly diplo cocci)	-
DM-85	4x10 ²	8x10 ⁴	Rod (short to medium)	-
DM-86	6x10 ²	1.6x10 ⁵	Rod (short)	-
DM-91	2x10 ²	7.2x10 ⁴	Cocci (diplo + short chain)	-
DM-92	3x10 ²	1.28x10 ⁴	Cocci (dispersed + chain forming)	-
DM-99	1.6x10 ³	4x10 ⁶	Cocci (dispersed + chain forming)	-
DM-105	2x10 ²	4.8x10 ⁶	Cocci (diplo + chain forming)	-
DM-106	5x10 ²	-	Cocci (mostly diplo + short chain)	-
DM-107	3x10 ²	2.8x10 ⁶	Cocci (diplo + short chain)	-
DM-120a	1x10 ²	-	Rod (medium size –thin)	<i>Clavibacter agropyri</i> (corynebacterium)
DM-135	3x10 ²	1x10 ²	Cocci (single + diplo + tetra)	-
DM-136	2x10 ³	2.2x10 ⁶	Cocci (slightly bigger, diplo + short chain)	-
DM-149	2x10 ²	-	Rod (very short)	-
DM-150	4x10 ²	-	Rod (single + short chain)	<i>Leifsonia aquaicat</i>

Only three isolates (Sample No. 29, 120a and 150) have showed successful grown on the OmniLog Plus ID System BUG Agar which is a prerequisite growth medium for identification System. No generous growth of all isolates was observed during one week incubation at 30⁰C to obtain enough culture for OmniLog Plus ID System analysis. According to the OmniLog Plus Identification, two different species, i.e. *Clavibacter agropyri* (*Corynebacterium*) for isolates No. 29 and 120a; and *Leifsonia aquatica* for isolate No 150 were identified with Low similarity. It was not possible to conclude the presence or absence of lactic acid bacteria by OmniLog Plus ID System in this study, since all of organisms that were suspected as lactic acid, were not grown on Bug agar. It could be research gap for others.

Generally, the overall fermentation trend and the total microbial spectrum of donkey milk samples are observed to be different from other dairy milk samples. In the first place, the distribution of bacteria resembles LAB was very low both in number and in kind in all analyzed samples as compared to the non-LAB groups and secondly, the role of this bacteria was also very insignificant unlike in the case of other dairy products fermentation in which they are known to play a very significant role. Thus in the current study, the organism resembles lactic acid bacteria were not capable of fermenting donkey milk, this indicates there are no fomenters bacteria, or most likely due to the effect of antimicrobial factor in donkey milk. Donkey milk does not have the normal fermentation trend but it showed separation of solid at the bottom and liquid phase at the top. It was incubated for 18 days to observe fermentation trend. During this incubation time pH level showed decreasing values. The pH of the donkey milk samples remained constant after 4.8 pH level for the entire time of the trials. In this case the microbial loads of some microorganism such as organisms seems lactic acid bacteria were increased while others such as gram negative and mold were decreased. Generally samples of donkey milk showed low microbial counts. The overall low microbial count may be linked to the high content of lysozyme, one of the milk components with is useful in bactericidal properties.

Chapter – 5

CONCLUSION AND RECOMENDATIONS

In this study, donkey milk resembles with human milk in basic composition of milk such as protein, lactose, vitamin C and physico-chemical characteristic such as density and acidity. But higher value in minerals composition was observed. The current result obtained for Abyssinia donkey milk closely resembles with existing literature value for donkey milk all most in all investigated parameters except Iron Unlike a previous assumption and as it occur in the cow's milk, donkey milk doesn't ferment under natural condition, but there is a drop in pH due to the action of microorganism. Samples of raw donkey milk also showed a low microbial counts further confirming its potential use as safe natural food for infant feeding. Moreover, most of the microorganisms that seem lactic acid bacteria during the isolation did not grow on MRS, M-17 and Bug agar, during OmniLog identification. This research has provided some data that donkey milk can somehow fill in the gap when breast milk can't be given to infant. But, before buying this recommendation further research need to be conducted in donkey milk sensory property, failure of lactic acid bacteria to grow on MRS, M-17 and Bug agar. Further investigation is also needed to know whether donkey milk can suppress the growth of potential pathogens. Based on the above conclusions the following points are recommended:

- More researches are needed on wider scale to characterize donkey milk properties.
- In the study area, the average milk yield of the morning milking of donkeys was found to be very low compared to cow's milk. Establishment of donkey-breeding centers has to be encouraged to develop, and select donkey breeds to improve milk production, and commercialize donkey milk.

Chapter – 6

REFERNCES

REFERENCE

- Adea woreda Livestock development and Marketing office (2000). Annual Report on Livestock development.
- Ahmed, M.M., Assegid, W., Jabbar, M.A., Ayele, S. and Belachew, H. (2003). Livestock marketing in Ethiopia: review of structure, performance and development initiatives. Socio-economic and policy Research Working paper 52.ILRI,Nairobi, Kenya.35pp.
- Alberse, R.C., Stapel, O., (2001). Structure of food allergens in relation to allergenicity: Pediatric. Allergy Immunol. 12 (Suppl. 14), 10–14.
- AOAC, (1980). Official Methods of Analysis 13th ed: Association of Official Analytical Chemists, Washington, DC.
- Bahna, S.L., Gandhi, M.D. (1983b). Milk hypersensitivity: II. Practical 295–301.aspects of diagnosis, treatment and prevention. Ann. Allergy 50.
- Biolog, inc. (2006). Omnilog Data Collection Software Identification System User Guide: Hayward, USA www.biolog.com
- Blench, R.M and MacDonald K (2000). The origins and development of African livestock archaeology, genetics, linguistics and ethnography: University College London Press, London, UK. ISBN 1-84142-018.
- Burns, J.P. (2001). HIV, Breastfeeding, and Infant Mortality: Weighing the Impact of the Non-evidenced-based WHO/UN AIDS. Recommendations in the Low-income, Low-resource Countries of Sub-Saharan Africa. Boston University, School of Public Health Department of International Health.
- Carroccio, A., Cavataio, F., Iacono, G. (1999). Cross-reactivity between milk proteins milk proteins of different animals: Clin. Exp. Allergy 29:1014-1016.
- Carroccio, A., Cavataio, F., Montaldo, G.D., Amico, D., Alabrese, L., and Iacono, G. (2000). Intolerance to hydrolyzed cow's milk protein in infants: Clinical characteristics and dietary treatment. Clin.Exp. Allergy 30:1597–1603.
- Central Statistical Authority, (2006). Ethiopia Demographic and Health Survey: Addis Ababa, Ethiopia.
- Central Statistical Authority, (2008). Statistical Abstract: CSA, Addis Ababa/ Ethiopia.

- Chandan, R.C., Parry, R.M., and Shahani, K.M. (1965). Lysozyme, lipase and ribonuclease in milk of various species: *Journal of Dairy Research*, 51, 606–607.
- Chiavari, C., Coloretti, F., Nanni, M., Sorrentino, E., and Grazia, L. (2005). Use of donkey's milk for a fermented beverage with lactobacilli: *Lait*, 85, 481–490.
- Chihara, G. (1992). Immunopharmacology of lentinan, a polysaccharide isolated from *Lentinus edodes*: its application as a host defense potentiator. *International Journal of Oriental Medicine*, 17, 57–77.
- Chye, F. Y., Abdullah, A., & Ayob, M. K. (2004). Bacteriological quality and safety of raw milk in Malaysia: *Food Microbiology*, 21, 535–541.
- Coppola, R., Salimei, E., Succi, M., Sorrentino, E., Nanni, M., Ranieri, P. (2002). Behavior of *Lactobacillus rhamnosus* strains in ass's milk: *Annals of Microbiology*, 52, 55–60.
- D'Alessandro, A.G., Martemucci, G. (2007). Influence of milking number and frequency on milk production in Martina Franca breed asses.
- Donnet-Hughes, A., Duc, N., Serrant, P., Vidal, K., Schiffrin, E.J., (2000). Bioactive molecules in milk and their role in health and disease: The role of transforming growth factor- β . *Immunol. Cell Biol.* 78:74-79.
- Doreau, M., Boulot, G. (1989). Methods of measurement of milk yield and composition in nursing mares: A review. *Lait* 69, 159-171.
- Dorea, J.G. (2000). Magnesium in human milk: *J. Am. Coll. Nutr.* 19. 210-219.
- Dugo, P., Kumm, T., Lo Presti, M., Chiofalo, B., Salimei, E., Fazio, A., Cotroneo, A., Mondello, L. (2005). Determination of triacylglycerols in donkey milk by using high performance liquid chromatography coupled with atmospheric pressure chemical ionization mass spectrometry: *J. Sep. Sci.* 28:1023-1030.
- Elaine W, Gunter, Wayman E., Turne Jane W., Neese, Ph.D., and David D., Bayse, Ph.D., (1976-1980). Laboratory Procedures used by the Clinical Chemistry Division, Centers for Disease Control, for the Second Health and Nutrition Examination Survey.
- El-Agamy, E.I. (2006c). Camel milk proteins are suitable for the nutrition of cow's milk protein allergic patients: *J. Food Chem.*, in press.
- El-Agamy, E.I., Abou-Shloue, Z.I., Abdel-Kader, Y.I. (1997). A comparative study of milk proteins from different species: II. Electrophoretic patterns, molecular characterization, amino acid composition and immunological relationships.

- Elisabetta S., Francesco F., Raffaele C., Biagina C., Paolo P., Giorgio V. (2009). Composition and characteristics of ass's milk.
- Ezedeen, H. (1998). The importance of donkey in a restocking programme in Eritrea: Ministry of Agriculture, Gash-Barka Zone, Eritrea.
- Fantuz, F., Vincenzetti, S., Polidori, P., Vita, A., Polidori, F., & Salimei, E. (2001). Study on the protein fractions of donkey's milk: In: Proceedings of the XIV A.S.P.A. Congress (pp. 635–637). Firenze, Italy.
- FAO, (1990). The technology of traditional milk products in developing countries: FAO Animal Production and Health Paper 85. FAO, Rome, Italy. 333 pp.
- FAO, (2005). FAOSTAT Data: <http://faostat.fao.org/site/409/Dektop>.
- Felleke, G. and Geda, G. (2001). The Ethiopian Dairy development policy: A draft policy document. Ministry of Agriculture/ AFRDRD/AFRDT Food and Agricultural Organization of United Nations/SSFF. Addis Ababa, Ethiopia.
- Getachew Felleke (2003). Milk and dairy products, post-harvest losses and food safety in sub-Saharan Africa and the Near East: Assessments Report on the Dairy Sub-sector in Ethiopia. Action Programme for the Prevention of Food Losses, FAO, Rome.
- Giosuè, C., Alabiso, M., Russo, G., Alicata, M.L., Torrisi, C. (2008). Jennet milk production during the lactation in a Sicilian farming system.
- Giuffrida, M.G., Cantisani, A., Napoletano, L., Conti, A. & Godovac- Zimmerman, J. (1992). The amino-acid sequence of two isoforms of alactalbumin from donkey milk is identical: *Biological Chemistry Hoppe-Seyler*, 373, 931–935.
- Goldman, A.S., Anderson, D.W., Sellers, W.A., Saperstein, S., Kniker, W.T., Halpern, S.T. (1963). Oral challenge with milk and isolated milk proteins in allergic children: *Pediatrics* 32, 425–443.
- Guo, H.Y., Pang, K., Zhang, X.Y., Zhao, L., Chen, S.W., Dong, M.L., Ren, F.Z. (2007). Composition physicochemical properties, distribution, and amino acid profile of donkey milk: *Journal of dairy Science*, 90:1663-1643.
- Groziak, S.M, Miller, G.D. (2000). Natural bioactive substances in milk and colostrums: effect on the arterial blood pressure system. *Br J Nutr* 84(1):119–25.
- Herrouin, M., Molle, D., Fauquant, J., Ballestra, F., Maubois, J.L., Leonil, J., (2000). New Genetic Variants Identified in Donkey's Milk Whey Proteins. *J. Protein Chem.*; **19**: 105.

- Horrobin, D.F. (2000). Essential fatty acid metabolism and its modification in atopic eczema: *American Journal of Clinical Nutrition*, 71, 367S–372S.
- Iacono, G., Carroccio, A., Cavataio, F., Montalto, G., Soresi, M., and Balsamo, V. (1992). Use of donkey's milk in multiple food allergy: *J. Pediatr. Gastroenterol. Nutr.* 14:177–181.
- Ibrahim, H. R., and Aoki, T. (2003). New promises of lysozyme as immune modulator and antimicrobial agent for nutraceuticals: *Foods and Food Ingredients Journal of Japan*, 208, 361–374.
- Jiao, B. H., Chen, J. H., Miao, W. M., Wang, L. H., Zhu, Y. P., & Miao, H. N. (2004). Identification and biological characterization of angiogenic and tumour growth inhibitors derived from *Sinica cetorhinus maximum* cartilage: *Marine Drugs*, 2,30–38.
- Kathy, M. and Zahra, A. (1998). Gender issues in donkey use in rural Ethiopia:
- Ketema, H. (2000). Dairy development in Ethiopia: In the role of village dairy co-operatives in dairy development: SDDP (smallholder Dairy Development Project) Processing's, MOA (Ministry of Agriculture), Addis Ababa, Ethiopia.
- Luthfor Ahmed, SK. Nazrul Islam, Nazrul Islam Khan and Syeda Nilufer Nahid (2004). Vitamin C Content in Human Milk (Colostrum, Transitional and Mature) and Serum of a Sample of Bangladeshi Mothers: Institute of Nutrition and Food Science, University of Dhaka, Dhaka-1000, Bangladesh. *Mal J Nutr* 10(1): 1-4.
- Malacarne, M., Martuzzi, F., Summer, A. and Mariani, P. (2002). Protein and fat composition of mare's milk: some nutritional remarks with reference to human and cow's milk. *International Dairy Journal*, 12, 869–877.
- Mohammad I., Hamayun K., Syed Shah H., Rasool K. (2008). Physicochemical characteristics of various milk samples available in Pakistan: Institute of Chemical Sciences, University of Peshawar, Peshawar-25120, Pakistan.
- Meftuh, A.B., Tapsoba, L.P. and Lamounier, J.A. (1990). Breast feeding duration on Ethiopian women in Southern California: *Journal of Tropical Pediatrics*, 36(4):203-4.
- Miranda, G., Mahe, M. F., Leroux, C., & Martin, P. (2004). Proteomic tools to characterize the protein fractions of Equidae milk: *Proteomics*, 4, 2496–2509.
- Morgante, M., Ranucci, S., Pauselli, M., Casoli, C., Duranti, E., (1996). Total and differential cell count in milk of primiparous Comisana ewes without clinical signs of mastitis: *Small Rum. Res.* 21:265-271

- Monti, G., Bertino, E., Muratore, M.C., Coscia, A., Cresi, F., Silvestro, L. (2007). Efficacy of donkey's milk in treating highly problematic cow's milk allergic children: an in vivo and in vitro study. *Pediatr. Allergy Immunol.* 18:258-264.
- O'Connor, C.B. (1992). Rural smallholder milk production and utilization and the future for dairy development in Ethiopia.
- O'Connor, C. B. (1995). *Rural Dairy Technology: ILRI Training Manual 1*. International Livestock Research Institute, Addis Ababa, Ethiopia.
- Oftedal, O.T. and Jenness, R. (1988). Interspecies variation in milk composition among horses, zebras and asses (*Perissodactyla: Equidae*): *J. Dairy Res.* 55:57–66.
- Pearson, R.A., Muir, C., and Farrow, M. (2006). *The Future of Working Equines: The Donkey Sanctuary*. Addis Ababa University, Ethiopia.
- Peeler, E.J., Green, M.J., Fitzpatrick, J.L., Morgan, K.L., Green, E. (2000). Risk factors associated with clinical mastitis in low somatic cell count British dairy herds: *J. Dairy Sci.* 83:2464-2472.
- Pieter Walstra, Jan T. M. Wouters, Tom J. Geurts (2006). *Dairy Science and Technology: Second Edition*.
- Salimei, E., Fantuz, F., Coppola, R., Chiofalo, B., Polidori, P. and Varisco, G. (2004). Composition and characteristics of ass's milk: *Anim. Res.* 53:67–78.
- Samia M.A., Abd Elrahman, Said Ahmad, Ibtisam E.M., El Zubeir., O.A.O. EL Owni and M.K.A. Ahmed (2009). Microbiological and Physicochemical Properties of Raw Milk Used for Processing Pasteurized Milk in Blue Nile Dairy Company (Sudan). *Australian Journal of Basic and Applied Sciences*, 3(4): 3433-3437, ISSN 1991-8178.
- Saxena, M.M., Katpatal, B.G. and Pandey, H.S. (1997). Study of milk constituent and their yield in Holstein-Friesian cows: *Indian Journal of Animal Production and Management* 13 (3):127-130.
- Schaafsma, G. (2003). Nutritional significance of lactose and lactose derivatives: In H. Roginski, J. W. Fuquay, and P. F. Fox (Eds.). *Encyclopedia of Dairy Science* (Vol. 3, pp. 1529–1533). London: Academic Press.
- Stephen, D. and Simon, P. (2006). Donkey milk holds the secret to skin health.
- Taha, N.M. and Kielwein, G. (1990). Pattern of peptide-bound and free amino acids in camel, buffalo and ass milk: *Milchwissenschaft* 45:22–25.

- Taylor, S.L. (1986). Immunologic and allergic properties of cow's milk proteins in humans: *J. Food Prot.* 49 (3), 239–250.
- Tesse, R., Claudia, P., Serena, B. and Lucio, A. (2009). Adequacy and tolerance to ass's milk in an Italian cohort of children with cow's milk allergy: Department of Biomedicine of the Developmental Age, Pediatric Unit "S. Maggiore", University of Bari, Bari, Italy.
- Van-den, B.J.C. (1988). Dairy technology in the tropics and subtropics: Pudoc, Wageningen, The Netherlands. 290 pp.
- Vincenzetti, S., Polidori, P., Fantuz, F., Mariani, P.L., Cammertoni, N., Vita, A., Polidori, F. (2005). Donkey's milk caseins characterization: *Ital. J. Anim. Sci.* 4(Suppl. 2):427-429.
- Vincenzetti, S., Polidori, P., Mariani, P., Cammertoni, N., Fantuz, F., Vita, A. (2007). Donkey's milk protein fractions characterization: *Food Chemistry* 106 (2008) 640–649.
- Webb B H, Johnson A H and Alford J A. (1974). *Fundamentals of dairy chemistry*: 2nd edition, AVI Publishing Company Inc, Westport, Connecticut, USA. 929 pp.
- White, C.H. (2001). Testing milk and milk products: In E. H. Marth and J. L. Steele (Eds.), *Applied Dairy Microbiology* (2nd ed., pp. 645–680). New York: Marcel Dekker.
- Yang, H. F., L. Ma, X. G. Zhao, and D. Q. Su. (2006). Studies on the development and exploitation of donkey milk: *Chin. Food Nutr.* 4:22–24.
- Young, W.P and George, F.W.H. (2006). *Handbook of Milk of Non-Bovine Mammals*: Blackwell publishing.
- Zegeye, Y. (2003). imperative and challenges of dairy production, processing and marketing in Ethiopia: In: Joger Y and Gebry G (eds), challenges and opportunity of livestock marketing in Ethiopia. Proceeding of the 10th Annual conference of the Ethiopian society of Animal Production (ESAP) held in Addis Ababa, Ehiopia, 22-24 August 2002. ESAP, Addis Ababa, Ethiopia. pp.61-67
- Zhang, X.Y., Zhao, L., Jiang, L., Dong, M.L. and Ren F.Z. (2008). The antimicrobial activity of donkey milk and its micro flora changes during storage: Beijing Functional Dairy Laboratory, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China.

Chapter - One

INTRODUCTION

Ethiopia holds the largest livestock population in Africa; which is estimated (excluding some regional stat) at about 43.1 million heads of cattle, 23.6 million of sheep, 18.6 million of goats, 0.62 million of camels, 34.2 million of chicken, 2.8 million of horses, 6.5 million of donkeys, 0.3 million of mules (CSA, 2008). However, milk production is among the lowest in the world; 1.54 liters (per cow per day) milk production from dairy cows (CSA, 2008). The contribution of the different livestock species to the total production of milk is about 81.2% from cattle, 6.3 % from camels, 7.9 % from goats and 4.6 % from ewes (CSA, 2008). The per capita milk consumption (16 kg/year), reported (Saxena *et al.*, 1997) has not changed much to date and is much lower than reports for Africa (27 kg /year) and developed world (100 kg/year), respectively. This clearly depicts the presence of a wide gap between the demand and supply of milk and milk products.

The dairy industry is not developed even when compared with east African countries like Kenya, Uganda and Tanzania. Regarding dairy production, the national milk production remains among the lowest in the world, even by African standards (Zegeye, 2003). The demand for dairy products is increasing as ever. Ethiopia is not self-sufficient in milk and a considerable amount of foreign exchange has to be spent on the import of dairy products. In Ethiopia one of the leading causes of infant mortality is malnutrition. Although milk is considered to be nature's perfect food for the growing infant, the ever rising scarcity and high cost of milk and dairying has made it impossible to meet the demand. Due to the highly perishable nature of milk and miss handling, the amount produced is subjected to high post-harvest losses. Losses of up to 20–35 % have been reported in Ethiopia for milk and dairy products from milking to consumption (Getachew, 2003). Although the dairy cow has been the predominant domesticated animal species for dairy production in developed countries, goats, sheep, water buffalo, yak, camel, and mare as well as some other minor mammalian species in which donkey is the one, have been domesticated and bred for milk production in regions of the world where the difficult environment required special adaptation and for which many of the non bovine mammals are better suited. While non bovine animals are an integral part of mixed-farming systems and hold several roles in Ethiopia, they provide the most needed animal power for the agriculture in addition to their production of milk and meat (Young, 2006).

The donkey is an important livestock species that is well adapted to the different environments. It serves for riding, as a pack animal for short distance transport, and as a draft

animal. A study showed that owning a donkey reduces the work load of women and at the same time increases time spent on other income generating activities (Ezedeen, 1998). A donkey gives about two liters of milk per day, over three milking, while a cow can churn out 40 liters in a single milking (Stephen *et al.*, 2006).

Donkey milk has long been praised for its unique nutritional quality and its closest similarity to human milk (Carroccio *et al.*, 2000). Consequently, donkey milk has been recently considered as a substitute for cow's milk protein due to its tolerance and nutritional adequacy in children with cow milk protein allergy and as safe, natural food in the condition where breastfeeding is not possible (Tesse *et al.*, 2009). Moreover, different studies also confirm donkey milk to be a natural product with good dietary and cosmetic properties (Carroccio *et al.*, 2000), used for probiotic purposes (Coppola *et al.*, 2002), with good palatability and optimization of the intestinal absorption of calcium which is essential for bone mineralization in infants (Schaafsma, 2003), and has high levels of linoleic and linolenic acid which are useful in the treatment of atopic dermatitis, tuberculosis and malaria (Horrobin, 2000). It has also a protective nature against viral fevers and flu-like symptoms such as asthma, runny nose and cough (Horrobin, 2000). However, there are no objective studies carried out to characterize the content and microbiological properties of donkey milk in Ethiopia for a potential use as safe and natural human breast milk supplement, substitute or its medical value.

Despite the presence of enormous products of animal origin, most are not consumed due to lack of awareness, religious, cultural taboos, and virtual absence of objective researches to bring neglected foods to human consumption. Moreover, women and children face huge and complex nutritional problems. One-fourth of Ethiopian women are malnourished, and approximately 50% of children less than five years of age are moderately or severely stunted (CSA, 2006). Analysis shows that malnutrition, even in its milder forms, accounts directly or indirectly for 53% of under-five deaths in Ethiopia (Burns, 2001). It is well known that the best nutritional option for newborns is their mother's milk, however, some infants may not be exclusively breast fed during the first months of life.

Accordingly, to alleviate the problem of infant mortality it is very important to improve optimal infant and young child feeding practices (Burns, 2001). While commercial milk replacements have become totally inaccessible due to cost, there is a need for another

substitute such as donkey milk which is claimed to be similar in composition and properties as human milk. This kind of research could pave the way to promote and raise awareness of the society on the importance and benefits of donkey milk and to break the social stigma associated with it. Moreover, this study might be the first of its kind in Ethiopia which would serve as a breakthrough in combating taboo against the donkey milk. Milk contents, on the other hand vary based on species, breed, locality, and stage of lactation. In order to use donkey milk for infant milk replacement, one needs to fully know the basic contents, and the biochemical characteristics. In this study it was hypothesized that donkey milk is equivalent in content and characteristics to human breast milk. Therefore, the general objective of the current study is to provide information about Abyssinian donkey milk from Adea Woreda that can potentially be used to replace or supplement human breast milk; while the specific objectives were:

- To characterize the nutritional content of Abyssinian donkey milk
- To analyze the microbiological properties of Abyssinian donkey milk

The significance of the study

The following are some of the significance of this study;

- This study could help as source of evidence and help create awareness to the community about donkey milk
- It will serve as an advocacy tool to minimize the negative attitude and taboo associated with donkey in general and donkey milk in particular
- The study also has add value for donkeys beyond the known value accustomed by society such as transportation, pack, riding and draft animal.
- The result of this investigation will help to show that donkey as an alternative or optional means for infant nutrition
- The finding of the study will have an importance as a substitution in helping children who are allergic to cow's milk protein. This is because donkey milk has close similarity with mother's milk which in all cases has no allergy to children
- It can serve as a starting or entry point for other researchers to investigate further on nutritional, medicinal and microbiological and cosmetic values of donkey milk
- Even though Ethiopia has the largest population of donkeys in Africa, this large resource has not been properly utilized. So, this study is hoped to pave a way for appropriate utilization of donkey's resource for milk and other purposes.

Limitation of the Study

This study has focused on Abyssinia donkeys which are mainly concentrated in the central parts of the county. So, this study is delimited only to the aforesaid types of donkeys and geographically to Adea woreda in Oromia region. In addition, the negative attitude and taboo associated to donkey milk hampered the researcher to get as many data as possible since owners are not willing to allow their donkeys for milking.

Other limitation of this study is scarcity of milk from donkeys. Given that most of the donkeys have not got enough and appropriate hay or food, it was a challenge to collect enough milk from sampled population at limited period of time and took several days in collecting milks for this purpose. Getting information about sampled donkeys was also another challenge since most of the owners don't have reliable information about their donkey's back history. This also limits the researcher to give full classifications of sample population.

Farmers or owners expectation for incentives from the researcher was also another area which can be considered as a limitation.

Chapter - 2

LITERATURE REVIEW

2.1. Milk production from donkeys

Donkeys are kept in Africa for four reasons: work, breeding, milking and eating. Of these, work is most important, and used mainly as pack animals, either for carrying loads or for riding. In arid regions they are used together with camels to pull water from deep wells. Less commonly they are used in traction, for example, pulling carts or plows, although both of these technologies are post-European introductions in sub-Saharan Africa (Kathy and Zahra, 2000).

Donkey's milk has been used for long period of time in the history of human kind. Dating back in 2500 B.C, Egyptians used this milk for different purposes. However, the first real scientific consideration was not given to this exceptional milk until the Renaissance period (Stephen *et al.*, 2006). One of the interesting historic report shows that Cleopatra, queen of Egypt, maintained her great beauty by bathing in donkey's milk and in same author report, the Romans also used donkey's milk for its therapeutic qualities. Like Cleopatra of Egypt, Poppaea, the wife of the emperor Nero, used this precious liquid to maintain the health and beauty of her skin (Stephen *et al.*, 2006). As indicated by Stephen and his colleagues, with the advice of doctors, François 1st king of France who was exhausted by wars underwent donkey's milk treatment and he found it very incredible and relieved him from stress and fatigue.

In the 19th century men and women of fashion from upper status society regularly consumed this precious beverage, but poor families saved this miraculous substance for a sick child or a weakened old person only. During this time, donkey's milk started to be regularly used in maternity hospitals and used to feed new-born (Tesse *et al.*, 2009). Furthermore, until the beginning of the 20th century, donkey's milk was marketed for the feeding of orphan babies, unhealthy children, ill people and the elderly. Tesse and colleagues, (2009) further elaborated that in the tradition of this use of donkey's milk, farms of donkeys were set up in Italy, France, Belgium, Switzerland and Germany during the start of 20th century. The success of these special farms that exploit donkey's milk confirms the product's ancestral reputation and all the significance of its vital properties. The modern day scientific community highly acknowledges that the value and importance of this heritage.

2.1.1. Values of donkey's milk

Donkey's milk is a milk which is rich in vitamins and has essential fatty acids and protein very similar to human mother's milk and it can be recommended in case of allergies to cow's, ewes and soya milk or simply when the mother wishes to give her child a safe, natural food (Tesse *et al.*, 2009). Similarly, donkey milk shares much similarity with mare milk in being low in total solids (8 to 10 %), protein (1.5 to 1.8 %) and being high in lactose (6 to 7 %). Its protein percentage has been characterized as having low casein nitrogen (47.3 %) as well as a high concentration of essential amino acids such as Val and Ly (Taha and Kielwein, 1990). Whey protein contents (36.9 %), where β -Lacto-globulin (LG) accounted for just 29.85 % (Salimei *et al.*, 2004).

As mentioned above when compared with milk from other species, donkey's milk presents a low dry material, fat and protein content but it is rich in lactose and calcium (Taha and Kielwein, 1990). The high lactose content of donkey's milk is revealed to be quite similar to that of a human's milk. This plays a fundamental role since it stimulates the intestinal absorption of calcium by having a positive influence on bone mineralization and since it stimulates the correct development of the nervous system in a child's first few months (Taha and Kielwein, 1990).

The level of fat in donkey milk ranges from 0.28 % (Chiavari *et al.*, 2005) to 1.82% (Ofstedal and Jenness, 1988). The lipid fraction of donkey's milk presents high levels of essential fatty acids. A fatty acid is qualified as "essential" when the organism can only produce them in small quantities. It is recommendable if human being in general and children in particular are provided with these essential acids throughout their daily diet or in the form of nutritional supplements, depending on type of the case (Guo, *et al.*, 2007). Some research findings depicts that the essential fatty acids present significant curative advantages as they contribute to the development of the brain and the retina. It is richer in long chain polyunsaturated fatty acids that also predominate in women's milk (52-54%), with a high percentage of linoleic acid (essential polyunsaturated fatty acid not synthesized by the organism); these polyunsaturated fatty acids seem to have immune-stimulant properties and cholesterol-lowering agents (Taha and Kielwein, 1990). Omegas 3 contained in donkey's milk play a major role with regard to the health of human

heart, since they help to fight against thrombosis (forming of blood clots) and heart rhythm disorders seem to be of precious help in preventing risks following a cardiac accident (Taha and Kielwein, 1990).

Different research reports show that donkey's milk has other importance apart from its nutritional value. It also presents a number of interesting characteristics in cosmetology (Vincenzetti *et al.*, 2007) because of its vitamins (A, B2, C and E) content and the vitamins in donkey milk enable to have several anti-ageing and skin regeneration effects which play an anti-oxidant role (Guo, *et al.*, 2007). Donkey milk has high vitamin C and in turn, vitamin C plays an anti-oxidizing role much sought after in cosmetics. It thus favors' the slowing down of the ageing process and speeds up the healing mechanisms. Vitamin E is recognized as being essentially an anti-oxidizer that slows down skin ageing and ensures the stability of cell structures (Iacono, *et al.*, 1992).

According to Iacono and colleagues, (1992) findings, it also contain a lot of unsaturated fatty acids such as Omegas 6 that are highly prized in cosmetics and contains a sufficient quantity of nourishing lipids to ensure the flexibility and well-being of the skin. Consequently the same authors found out that essential fatty acids which is rich in donkey's milk helps the skin to absorb vitamins as it allows the skin to become elastic and prevent certain skin diseases (Iacono, *et al.*, 1992). Mineral salts contained in donkey's milk allow the skin to be cleaned. They get rid of dead cells, thus, leaving room for cells that live on the skin's surface and influence cell growth (Vincenzetti *et al.*, 2007).

Another most important and interesting part of donkey's milk consumption for human being is its hygienic value. Its pH is close to neutrality (7.2), but higher than that of cow's milk (pH = 6.3to 6.8). Its strong lysozyme content and its relative shortage of casein and phosphate explain, in all likelihood, the low microbial contamination of donkey's milk (Zhang *et al.*, 2008). Indeed, the quantity of bacteria found is very low: 4.46 log cfu/ml. These values only increase very little at stages of advanced lactation: 5.2 log cfu/ml. The rate of maximum bacteria tolerated by international directives for human milk consumption is not an entry barrier, far from it. This amount even represents an asset for donkey's milk in the context of industrial processing of a microbiologically sensitive product. Fresh donkey's milk represents ideal nourishment in the

context of minimum risk food. The high lysozyme content, which has a selective action vis-à-vis potentially pathogenic micro flora in the gastrointestinal tract of the new-born. The high lysozyme content of donkey's milk makes this food naturally "sterile" with an extremely low bacterial burden even after being kept in the fridge for 15 days with no preventive heat treatment (Zhang *et al.*, 2008).

Table 1. Physicochemical composition of donkey and human milk obtained from literature

Milk characteristic	Donkey milk			Human milk		
	N	Mean	SD	N	Mean	SD
PH	70	7.18	0.13	20	6.89	0.08
Density	150	1.03	0.02	20	1.027	0.011
Acidity%	-	-	-	20	0.072	0.011
TSS %	150	8.91	0.31	20	10.71	0.11
Fat %	70	0.38	0.12	20	2.1	0.15
Protein %	70	1.56	0.14	20	1.94	0.14
Lactose %	70	6.33	0.17	20	6.45	0.08
Vitamin C	-	-	-	26	3.06	0.67
Calcium	24	676.7	284.32	20	340	3.5
Magnesium	24	58.46	8.43	20	30	0.35
Iron	24	1.15	0.51	20	0.4	0.00
Zinc	24	1.22	0.52	20	2.3	0.16
Phosphors	6	487.0	-	20	160	0.85

Source: Elisabetta *et al.*, 2009; Dorea, 2000; Salimei *et al.*, 2004; Guo, *et al.*, 20007

2.1.2. Yield and quality of donkey milk

The small cisternal size, the structure of mammary gland in Equidae affects the need for a greater number of daily milking (Doreau and Boulot, 1989). The asinine species is considered a seasonal polyestrous one, but the latitude in which the farm is located can greatly influence the reproduction cycle; considering a recent investigation in the south of Italy, specifically in Sicily, the local latitude determined small photoperiod oscillations between the different seasons, and under these conditions the donkeys have a continuous reproductive cycle, the same was observed

also in sheep (Giosuè *et al.*, 2008). In Sicily it is in fact possible to obtain donkey milk throughout the year by adequately planning the breeding seasons; results showed that donkeys foaling in winter and in summer produced more milk than those foaling in the other seasons. Considering the different milk yield obtained when donkeys were milked twice a day, in the morning and in the afternoon (Salimei *et al.*, 2004), the average milk yield of the morning milking was found to be statistically lower than that observed for the afternoon milking, that is 549.2 mL vs. 949.3 mL respectively (D'Alessandro and Martemucci , 2007) have investigated on the effects of daily milking number and frequency on donkey milk production and the results obtained in that study demonstrated that the highest milk yield corresponded to three milking per day every three hours, while daily milking regimen of six milking per day did not increase milk production and had a negative influence on the health of the mammary gland.

2.1.3 Production of donkey milk in the world

Even though all mammals produce milk, it is only few species which produce milk that is utilized by human beings. The most important mammals which produce milk are cattle, buffalo, sheep, goat, horse, donkey and camel. The quantities of milk produced by various species differ markedly and individual animals also show wide variations in production within one species, mainly depending on domestic purpose, breed and genetic quality, environmental conditions, physiological conditions and level of management. Mares, donkeys and camels are principally kept as draught, pack or riding animals. Milk production is secondary and milk is only incidentally consumed, although in some areas the milk of these animals is highly appreciated and may be used for the manufacture of special fermented milk (Van Den Berg, 1988).

The advantages of donkey milk have attracted considerable interest in its use for human consumption today. However, donkey breeding is so disperse that the milk yield is very low. With almost 8 million head of donkey in 2004, China has the largest donkey stock worldwide, followed by Ethiopia (FAO, 2005). Donkeys are primarily raised in Northwest China in the Xinjiang and Shanxi provinces. In the last several years, the Xinjiang province has strived to establish donkey-breeding centers, leading to a great improvement in the yield of donkey milk.

Annual milk production has reached up to 40,000 metric tons, ranking first in world (Yang *et al.*, 2006), and has helped make donkey milk processing possible.

2.2. Milk production from other non bovine mammals

No one can deny the fact that cows are the primary dairy animal species in many countries to provide humans with nutritious food through the abundance of their lacteal secretion. Goats and other minor dairy species will never be able to compete with cows in terms of volume of milk production. However, the contribution of milk from other domesticated dairy species to the survival and well being of people around the world is immense and invaluable, especially in areas where cows have difficulty to surviving. Milk of non bovine animals is not only for people in the developing world who want and need to better their food supply quantitatively and qualitatively, but is especially for people in the developed world who may have medical needs for alternative foods and treatment, or for a higher- quality menu.

Sheep have been milked for millennia, but mainly as part of triple-purpose breeding for fiber and meat production besides milk. Therefore official statistical records of dairy sheep populations, and sheep milk production and processing, are hard to find. Sheep milk has unique composition and is ideally suited for yogurt and cheese production. Buffalo milk is important in Asian countries mainly, but the distribution of buffalo populations and interest in buffalo milk products is spreading. India has supported officially significant research with dairy buffaloes. Buffalo milk is popular in many traditional products, which are not well known in Western countries. Mare milk is another Asian uniqueness, with much tradition in some countries, but also with some good justification as an alternative to cow milk and treatment for humans with debilities. Camel milk also has unique compositional differences from cow milk, among which is an absence of beta-lacto globulin, which makes it more similar to human milk (Young, 2006).

Reindeer milk has received new interest from researchers in the North, mainly because of its highest level of milk composition and the unique adaptation of this ruminant mammal to the very harsh climate. Protein in reindeer milk is about three times the level in cow milk, but lactose is uniquely less. Sow milk is of considerable academic and research interest because of the

physiological similarity of this monogastric to human milk secretion. Nutritional, physiological, and biochemical research data on sow milk can be effectively utilized and applied to related situations in human metabolism, health, and medicine (Young, 2006).

Additional other minor species milks such as moose, musk, donkey, zebra, and pony are produced for human consumption only at certain specific regions of the world, very little data is available due to the extremely limited studies that have been conducted on such rarely domesticated minor species mammals. Academically, there should be much stimulation from the different uniqueness among those species (Young, 2006). The technical and popular literature abounds with publications about the world of cow milk, while milk of other mammals has given little attention. Non-bovine mammals have an important nutritional, economic, and social role. Non-bovine mammals includes: goats, sheep, buffaloes, mares, camels, donkey, yaks, reindeer, sows, llamas, and humans. Focus on donkey milk is the initial motivator for this paper, because of some unique characteristics of donkey milk compared to cow milk. Donkey milk has been used successfully in cases of cow milk allergies and by patients with various metabolic and gastrointestinal ailments. Donkey milk proteins can differ genetically from cow milk proteins (Carroccio, 1999).

2.3. Potential of donkey milk production in Ethiopia

The importance of donkeys in the past as well as to date has not been considered a significant component of the development process. Leave alone donkey's milk, beyond for the transportation purpose, donkey is considered one of the insignificant animal in Africa in general and in Ethiopia in particular. The attitude of the society towards donkey is not positive. For many of the institutions promoting 'development', the use of donkeys has been considered an indicator of backwardness and underdevelopment. Traditional attitudes to donkeys have also been quite negative and in some instances have inhibited the spread of donkey use. This attitude has led to a loss in the traditional knowledge relating to donkeys and to a lack of investment in the research and development of donkey issues. Field observations however indicate that this may be changing. In parts of Ethiopia, farmers observed that in periods of significant food insecurity, donkeys were more important than oxen (Kathy Marshall and Zahra Ali, 2000). The taboo and

negative attitude associated with donkey has led to neglect and less care. Now the importance of the donkey is finally being appreciated, a positive change in their image is possible. This should lead to a greater acceptance and awareness of this species as an important resource.

Since Ethiopia has the largest donkey population 6.5 million (CSA, 2008) and largest density in the world (27 donkeys per 100 people; Pearson *et al.*, 2006), it is possible to enhance donkey milk production by establishing donkey-breeding centers, which leads to an improvement in the yield of donkey milk. Considering its unique nutrient profile and economic potential, donkey milk could be exploited to fulfill the nutritional requirements of the people of Ethiopia particularly children. However, nothing is known about domestic donkey milk in Ethiopia. Therefore, there should be a need to promote the use of donkey milk in Ethiopia and to develop specialized center of donkey breeding particularly in those area where they are predominantly found and potentially productive.

2.4. Properties of donkey milk

2.4.1. Fermenting activity

The possibility of producing a fermented beverage from donkey's milk using the probiotic bacterial strains help to understand the microbiological properties of donkey milk which has a low microbiological load and an elevated content of lysozyme. The bacterial strains employed for fermentation had a good growth capacity in donkey's milk only after an initial adaptation phase. An extremely high percentage of viable bacteria were found in the final beverage. Likewise, the activity of lysozyme was virtually unchanged with respect to initial value. Technology can be proposed for production of a fermented beverage from donkey's milk that can be utilized by small producers. This would allow the production of a beverage that would be well accepted by consumers interested in a product with favorable therapeutic properties integrated with probiotic bacteria (Chiavari *et al.*, 2005).

Enzymes in donkey milk possess some unique characteristics such as bactericidal property which makes donkey milk different from other mammals' milks (Malacarne *et al.*, 2002). Milk is a good

medium for the growth of many microorganisms, since it contains many necessary nutrients and provides a suitable physical environment (White, 2001). In another way, milk supplies an array of defense factors in the form of lactoperoxidase peroxide system (LPS), lactoferrin, lysozyme, immunoglobulins and free fatty acids that protect it from different microbial infections.

2.4.2. Antimicrobial activity

Report from different professional in the area indicate that donkey milk contains several antimicrobial factors such as lactoferrin, lactoperoxidase and lysozyme, that have the ability to kill and inhibit a broad spectrum of bacteria in the digestive tract to reduce the incidence of gastro-intestinal infections or by defending against infections of the mammary gland. Milks of different species contain different amounts of the various antimicrobial factors.

Lactoferrin presents in milk are synthesized by the epithelial cells of the mammary gland. Human milk is particularly rich in lactoferrin. The concentration in the colostrums ranges from 1 to 16 g/l where as in the mature milk is 1-2 g/l. In bovine colostrums lactoferrin ranges from 0.2 to 5 g/l but decreases in mature milk to 0.1-0.2 g/l (Miranda *et al.* 2004; Greppi and Roncada, 2005), in donkey's milk the level of lactoferrin is reported to be two times higher than that of bovine milk (Malacarne *et al.*, 2002). Lactoferrin is an iron-binding protein and involve in the hydrolysis of glycosidic bonds of mucopolysaccharides in bacterial cell walls. Donkey milk is also known to contain a low microbial content (Chiavari, 2005; Coppola, 2002). This can be attributed to the antimicrobial substances present in donkey milk.

Other authors reported the donkey's milk has two variants of lysozyme that are lysozyme A and B that differ for three amino acid substitutions at different position (Herrouin *et al.* 2000). Lysozyme is an enzyme presents in the milk of some species, in human milk the concentration is 0.2 g/l, in bovine and in goat milk is nearly undetectable, in mare's milk is 1.1 g/l (Miranda *et al.*, 2004). In particular, the concentration of lysozyme in donkey milk reaches the level of 4 g/ L, (Guo *et al.*, 2007). The high lysozyme content of donkey milk may be responsible of the low bacterial count reported in literature and also makes this milk suitable to prevent intestine infection to infants.

In addition, lysozyme has other physiological functions, including inactivation of certain viruses, immuno- regulatory activity, anti-inflammatory and antitumor activity (Ibrahim & Aoki, 2003). Several studies have indicated that lysozyme derived from hen egg white, marine bacterium and shark cartilage possesses effective angiogenic inhibition and anti-tumor activities (Jiao *et al.*, 2004; Sava, 1989; Ye, Wang, *et al.*, 2008). The anti-tumour mechanism can be summarized into two different categories: enhancement of immunity against tumours (immuno-enhancing activity), through stimulating the production of immuno-enhancing and tumoricidal cytokines and direct anti-tumors activity to induce apoptosis of tumors cells (Zhang, *et al.*, 2006).

The active components of donkey milk had an accumulation effect on the G0/G1 and G2/M phases of A549 cells, i.e., the proliferation of A549 cells were arrested in the G0/G1 and G2/M phases of the cell cycle. This observation explains the anti proliferative effect of donkey milk. Several reports have shown that cell proliferation and differentiation were specifically controlled in the G1 phase (Hall & Peters, 1996). The significant suppressive effect of donkey milk on the proliferation of tumor cells is partly attributed to the promotion of differentiation of A549 cells into normal cells. According to Chihara (1992), donkey milk could induce a marked increase in cytokine production from lymphocytes and macrophages. These cytokines are contributory to an immune response and result in maturation, differentiation, and proliferation of immuno-competent cells for defense mechanisms of the host which explains the possible immuno-enhancing mechanism of the anti-tumour activity of donkey milk.

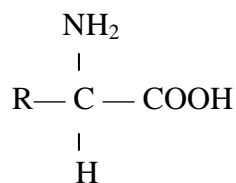
The concentration and activity of each antimicrobial system/substance show wide variation among species (Chandan *et al.*, 1965). Donkey milk is reported to have stronger inhibitory activity than that of any other species.

2.4.3. Protein composition

It is clearly known that proteins are indispensable for the growth, development and maintenance of all cells in the body. Various research outcomes found out that the value of milk proteins depends primarily on their content of some nine essential amino acids which cannot be

synthesized by the body. Cow and human milk contain all the essential amino acids required for human infants. Fortunately, both cow and human milk are easily digested and the amino acids are readily absorbed. Proteins are essential to all life process and perform a variety of functions in living organisms ranging from providing structure to reproduction. The natural function of milk proteins is to supply young mammals with the essential amino acids required for the development of muscular and other protein-containing tissues, and with a number of biologically active proteins, e.g. immunoglobulin's, vitamin-binding protein, metal-binding proteins and various protein hormones.

In the history of human kind, milk proteins represent one of the greatest contributions of milk to human nutrition. Proteins are polymers of amino acids that means only 20 different amino acids occur regularly in proteins. The amino acids have the following general structure (O'Connor C B, 1995).



The properties of the acid are affected by different radicals of amino acid and in this case each amino acid has different radicals. The content and sequence of amino acids in a protein affect its properties. Some proteins contain substances other than amino acids and are called conjugated proteins; these include Phospho-proteins in which phosphate is linked chemically to the protein, e.g. casein in milk and phosphoproteins in egg yolk. Lipoproteins which are combinations of lipid and protein are excellent emulsifying agents. They are found in milk and egg yolk. Chromo proteins which have a colored prosthetic group and include hemoglobin and myoglobin (O'Connor C B, 1995).

About 95% of nitrogen in milk is in the form of protein. Multiplication of the nitrogen content by a kjeldhal factor of 6.38 is generally accepted to give the protein content in milk and milk products, although the kjeldhal factor varies among milk protein (Pieter, 2006). Normal bovine milk contains about 3.5% protein (Weeb, *et al.*, 1974). The concentration changes significantly

during lactation, especially during the first few days post-partum; the greatest change occurs in the whey protein fraction. The young of different species are born at very different states of maturity, and, consequently, have different nutritional and physiological requirements. These differences are reflected in the protein content of the milk of the species; the protein content of milk is directly related to the growth rate of the young of that species, reflecting the requirements of protein for growth.

The properties of many dairy products, in fact their very existence, depend on the properties of milk proteins, although the fat, lactose and especially the salts, exert very significant modifying influences. Casein products are almost exclusively milk protein while the production of most cheese varieties is initiated through the specific modification of proteins by proteolytic enzymes or isoelectric precipitation. The high heat treatments to which many milk products are subjected are possible only because of the exceptionally high heat stability of the principal milk proteins (Van den Berg J C T. 1988). Specifications for many dairy products include a value for protein content. Changes in protein characteristics such as insolubility as a result of heat denaturation in milk powders or the increasing solubility of cheese proteins during ripening are industrially important features of these products.

Initially, it was believed that milk contained only one type of protein but about 100 years ago (Dairy Science and Technology, 2006) it was shown that the proteins in milk could be fractionated into two well-defined groups. On acidification to pH 4.6 (the isoelectric pH) at around 30°C, about 80% of the total protein in bovine milk precipitates out of solution; this fraction is now called casein. The protein which remains soluble under these conditions is referred to as whey or serum protein or non-casein nitrogen (FAO, 1990). The ratio of casein : whey proteins shows large interspecies differences; in human milk, the ratio is 40 : 60, in equine milk is 50: 50 while in the milks of the cow, goat, sheep and buffalo it is 80 : 20.

In addition to the caseins and whey proteins, milk contains two other groups of proteins the protease-peptone fraction and the non-protein nitrogen fraction. The heat-denaturable whey proteins represented the lacto globulin and lactalbumin fractions and designated the remaining 20% protease-peptone. The protease-peptone fraction was precipitated by 12% trichloroacetic acid

but some nitrogenous compounds remained soluble in 12% TCA and were designated as non protein nitrogen (FAO, 1990).

2.4.4. Other properties of donkey milk

Donkey milk hygiene should also be evaluated, as recently stated by Conte (2008). The EC Regulation n. 853/2004 allows the sale of ass' milk being these animals included in the "other milk producing species". According to this EC Regulation, the donkey milk should have a total bacterial plate count of less than $<1,500,000/\text{ml}$ at 30°C . But, if this milk is assigned to infants or elderly human beings, it's absolutely necessary that, besides the nutritional requirements, the sanitary aspects will be evaluated too. Conte (2008) has already discussed the European indications as inadequate for the control of milk quality in donkeys, demonstrating that in presence of some milk bacteria (*S. aureus*, *S. intermedius*, *S. dysgalactiae*) there are no evidences, in donkeys, of clinical signs and/or increased somatic cell count (SCC). The dairy industry is currently focusing on SCC as marker of both milk quality and udder health, and the important role of SCC in the pathogenesis of mastitis is well described (Morgante *et al.*, 1996). Peeler *et al.* (2000) have demonstrated that sometimes, in and «infected» milk (in cows, goats and ewes), the changes does not regard only the number of cells but the type of them. Furthermore, as the severity of inflammation associated with the mammary gland disease increases, the protein composition of milk changes approaching that of blood (as a consequence of increased permeability of the blood mammary barrier or intramammary synthesis). Many of these proteins are the so called «acute phase proteins» (APP). In bibliography no data exist on the differential leukocyte cell count (DCC) for donkey milk, neither on the acute phase proteins (APP) fluctuations during an entire lactation period and/or during mastitis. The peculiarity of donkey milk together with the potential consumers require indeed an accurate evaluation of milk quality and udder health that should pass through the evaluation of DCC, together with the acute phase proteins (APP) assessments.

2.5. Nutritional characteristics of donkey milk

As being repeatedly mentioned above, milk is one of the most common causes of food allergies among children under one year of age. No specific therapy exists for this allergy, and thus the only feasible response is to avoid consumption of milk and derived products. Studies conducted on the serum of children with hypersensitivity to milk have shown that caseins are the proteins with the greater allergenic potential. However, in some cases, children have also shown hypersensitivity to the B-lactoglobulines and to the α -lactalbumins.

Various and dependable research reports show that when food intolerance is diagnosed in an infant, it is often necessary to impose a period of total parental feeding, followed by breast feeding, considered the most correct method of re-feeding; when human milk cannot be given, alternative food sources must be sought. Soy or hydrolysed formula milks are good substitutes of human milk and also permit the children to grow up well, but they have not a good taste, therefore in some cases are refused by the infants. Furthermore it has been reported some cases of infant with intolerance to hydrolysed cow's formula or goat milks. Some findings conducted by Fantuz *et al.*, 2001; Salimei *et al.* 2004; Vincenzetti *et al.*, 2005a have demonstrated that donkey milk could substitute breast feeding in infants affected by severe Ig-E mediated milk allergies. In this case donkey milk is not only useful, but also safer than other types of milk. In fact donkey's milk composition in lipid and protein fraction, mineral content and lactose content is comparable to human milk. The new, current findings and analysis has come up with a solution for cow milky allergy since donkey milk substitutes and also more emphasis needs to be given to the protein fractions. It is considered to be a good alternative to infant formulas, since its nutrient composition, particularly the proteins, are very similar to those of human milk (Monti *et al.*, 2007).

2.5.1. Donkey milk total protein

Donkey's milk total protein content is the lowest (13 - 28 g/l); therefore it does not produce an excessive renal load of solute compared with that of bovine milk that contain 32 - 34 g/l (Salimei *et al.*, 2004) but it is very close to the values for human (9 - 15 g/l) and mare's milk (Doreau and

Boulot, 1989). In goat milk the total protein content is closest to that of bovine milk. The ratio caseins/whey proteins depend on species and it is high in rapidly growing mammals (lambs and calves). According to Saliemei and colleagues (2004), in donkey's milk the total casein content is about 8.7 g/l, which is very close to the value found in the human milk. Alpha s_1 - and B-caseins of caseins are identified. K- and Y-caseins can't be found probably due to low amount. The proteins that remain soluble in milk serum after precipitation of caseins at pH 4.6 at 30°C are whey protein. Similarly, its content in donkey milk is 1.8 mg/ml (Salimei *et al.*, 2004) and they are also variable at different stages of lactation periods.

About 35–50% of protein in donkey milk protein fraction is whey protein, while in cow's milk, it is only 20% (Herrouin *et al.*, 2000). A-lacto albumin and B-lacto globulin are of particular interest among whey proteins, this is because they are considered as the principal milk allergens in neonates and children (Carroccio *et al.*, 1999), but b-LG is absent in the human milk. Fantuz *et al.*, 2001, demonstrates that α -lactalbumin, b-lacto globulin and lysozyme are the most important whey proteins in equid milks. A-LA in donkey milk has two isoforms, called A and B, with different isoelectric points (Giuffrida, *et al.*, 1992) and it has a specific physiological function in the mammary gland, it interacts with the enzyme B-14-galactosyl transferase to form the lactose synthase complex and results in the modification of the substrate specificity of B-14-galactosyl transferase allowing the formation of lactose from glucose and UDP-glucose. The tightly bounded Ca^{++} that is contained in α -lacto albumin that influence the structure of protein does not affect lactose synthesis. According to studies carried out by Greppi and Roncada, 2005, the concentration of α -LA found in bovine milk is at an about 1.2-1.5 g/l (similar to the value in human milk (1.6g/l) and goat milk (1.2g/l) where as it is estimated 3.3 mg/ml in mare milk (Miranda *et al.* 2004; Greppi and Roncada, 2005). In the bovine milk, the concentration of α -LA is higher at the beginning of lactation and decreases near the end of lactation. This decrease is correlated to the decrease of the lactose concentration at the end of the lactation.

Donnet, 2000, discovered the existence of several variants of B-lacto globulin in donkey's milk which mainly exist as A or B variant. Accordingly there are three genetic variants of b-LG are found, these are: I-like b-LG with three amino acid substitutions, while the other two are similar to b-LG with two amino acid exchanges (Herrouin *et al.*, 2000). Structurally b-lacto globulin is

similar to retinol-binding protein and lipocalins. This may suggest that B-LG may have a role in the transport of fatty acids and vitamin A.

2.5.2. Nutritional sufficiency of donkey milk

Professionals in the field agreed and universally reached on consensus that breast milk is the perfect food for newborns with healthy mothers who have appropriate nutritional stores (Carroccio, 1999). Due to many reasons and health problems, in some cases babies cannot be breast-fed. So, it is necessary to find out what kind of milk and from which species or animal is the most suitable for babies. This is one of the important issues that this review of literature tried to address. One of the challenges this time around Cow's milk is allergy which is a common disease which causes sickness of infancy. Infant formula, an industrial milk product usually based on either cow or soy milk, strives to be an adequate artificial substitute for natural breast milk. However, since breast milk cannot be fully reproduced and sometimes babies can suffer from allergy when fed infant formula so that donkey milk can fill this gap (Carroccio *et al.*, 2000).

Researchers in the field believe that the positive results from the pediatrics trials are due to the particular composition of donkey milk protein fraction. According to Donnet, *et al.*, 2000, in addition to the traditional nutrients of the diet, bioactive molecules in functional foods, including milk, are clearly important in providing hormonal, antibacterial, probiotic or pharmacological support. In this regard, the properties of donkey milk could be due to its probiotic activity, high lactose content, (Coppola *et al.*, 2002) the presence of trophic and protective factors, immunoglobulin, peptides with antibacterial and hormonal activity, its excellent nutritional value and easily digestibility (Miranda *et al.*, 2004), in addition to its unique nitrogen composition, with a low casein-to-whey protein ratio makes it a good alternative to human milk in infant nutrition. Some of these protective factors, often characterized by inter specific activity, are also present in dairy cow's milk, but may not survive the technological processing used during the preparation of infant formulas. It is also important to consider the high lysozyme content of donkey milk in relation with its probable role in preserving the intestinal ecosystem from potentially harmful

microbes. The use in dermatology, which is well known since ancient Rome is also efficacious in smoothing skin and scalp inflammations.

Substances with bioactive properties are also in the category of lipids increasing importance is now attributed to the milk content of mono-di-and triglycerides, free fatty acids, phospholipids, glycolipids and steroids along with minor constituents, like carotenoids, vitamins and lipoproteins (Dugo *et al.*, 2005). Bearing in mind the fatty acids compositions of milk, most interest is actually addressed to polyunsaturated fatty acids, essential in immune-modulating activities, growth (Gill *et al.*, 2000) as well as in the development of the neonatal nervous system. In this regard, results of a study conducted by Doreau *et al.*, 2002, Shows the interesting free fatty acids profile of donkey milk, with a lower total saturated fatty acids content, an average higher level of total unsaturated fatty acids. Those experimental observations could support the positive contribution of donkey milk to an efficient cerebral growth and the development of a normal and complete immunitary system in the neonate (Carroccio *et al.*, 2000).

Overall, the good palatability of donkey milk and the very close chemical composition compared to human milk seem to encourage the use of this product in infant feeding especially in cases of multiple food hypersensitivity, lactose intolerance. Although the mechanism of this tolerance has not yet been fully clarified, it is reasonable to hypothesize that the reduced allergenic properties of donkey's milk can be related to structural differences of its protein components with respect to bovine milk. The high lactose content, the characteristic protein profile with a lower casein content (50 %) with respect to cow's milk (80 %) and the relevant percentage of essential amino acids make donkey milk a potential new dietetic food and a promising breast milk substitute.

2.5.3. Use of donkey milk as human milk substitutes

In any ratio and structure of milk constitutes, it has been found out that the composition of human milk is quite different from that of other mammalian milks. The protein content in human milk is closer to that of donkey and mare milk. Conversely, it is lower than in milk of ruminant dairy animals, cows, buffalo, camel, goat, sheep (El-Agamy *et al.*, 1997). The ratio of casein within total protein is lower in human milk, because whey proteins (soluble proteins) are higher than in

cow, buffalo, and sheep milk, whereas they are at similar level in donkey and mare's milk (El-Agamy *et al.*, 1997). This condition gives human milk the special property of forming a soft curd during digestion in the infants' gut. The softness of the curd is due to the lower ratio of soluble calcium. This condition may explain, why in many parts of the world mare and donkey milk are used as human milk substitutes for bottle fed infants (El-Agamy *et al.*, 1997). On the other hand, both cow and buffalo milk gives a hard curd, which of course is preferred in cheese making.

Prior to using for baby feeding, dilution of bovine milk with water must be practiced for safe nutrition, especially for very young babies. In contrast, human milk proteins are different in their composition and structure from those of the milk of other species. It is known, that the major whey proteins of bovine milk are β -lg with 55% of total whey proteins, alpha lactoalbumin with 20%, and blood serum albumin with 7% (Taylor, 1986). These proteins differ in their types and ratios between goat, sheep, cow, camel, human, buffalo, mare and donkey milks (El-Agamy *et al.*, 1997). Human milk is free of beta lacto globulin, one of the major allergens in cow milk, similar to camel milk, which also has no beta lactoglobulin (El-Agamy, E.I., 2006c). On the contrary, beta lactoglobulin is a major whey protein in cow, buffalo, sheep, goat, mare and donkey milk (El-Agamy *et al.*, 1997). Caseins in the milk of these species differ in fraction number and their electrophoretic behavior on polyacrylamide gel, amino acid composition and their peptide mappings (El-Agamy *et al.*, 1997).

Donkey milk has several advantages over cow's milk for human consumption. Recently, cow milk protein allergy has drawn the attention of researchers over the world. It is an altered or abnormal reaction. Such a reaction may occur when there is contact between a foreign protein "an allergen" and body tissues that are sensitive to it. The allergy may reach the tissues by direct contact with the skin mucous membranes or through the blood stream after absorption. Allergic reactions have been classified into two types: immediate reaction type and the delayed reaction type. Cow milk allergy is clinically an abnormal immunological reaction to cow milk proteins, which may be due to the interaction between one or more milk proteins and one or more immune mechanisms, and resulting in immediate IgE-mediated reactions. On the other side, reactions not involving the immune system are defined as cow milk protein intolerance. CMA occurs in some infants after ingestion of an amount of cow milk (Goldman *et al.*, 1963).

Hypersensitivity to cow milk proteins is commonly one of the main food allergies and affects mostly infants and also adults in infrequent cases. Cow milk protein allergy may be very severe in some cases. Different clinical symptoms of milk allergy have been established. The diagnosis of milk allergy differs widely due to the multiplicity and degrees of symptoms, and can be achieved by skin or blood tests. Cow milk contains more than 20 proteins that can cause allergic reactions. Casein fractions and b-lacto globulin are the most common cow milk allergens (Aalberse, 2001).

With the introduction of new and different procedures, some possibilities are adapted in reducing the allergen-city of milk proteins. Different attempts have been made and various technological processes have been applied in order to have better use of cow milk in infant formulae. These are heat treatment, infant formula, enzymatic treatment, extensively hydrolysed formula, amino acid-based formula and soy formula. However, this is a bit costly and requires various procedures, so that using, camel, mare or even soy milk and donkey milk as alternatives in cases of cow milk allergy. Milk from these species can be considered hypoallergenic. Allergies to milk proteins of non-bovine mammals have also been documented due to cross-reactivity between cow milk proteins and their counterpart in other species, and even between goat and sheep caseins. Genetic polymorphisms of milk proteins play an important role in eliciting different degrees of allergic reactions. However, therapeutic benefits vary with the degree of severity of the allergy (El-Agamy, 2006c).

Chapter - 3

MATERIAL AND METHODS

3.1. Study area and study animals

Adea woreda is found in Eastern Shewa zone of Oromia regional state in Ethiopia. It has 27 rural kebeles and each has 2-4 villages. Big towns like Bushoftu are found in this woreda. For this specific study, three rural kebeles namely Garaboru, Babogaya and Hiddi were selected. The total human population in these kebeles is about 17600. With respect to livestock population, these kebeles have more (Table 1) Cattle, Sheep, Goat and Donkey population than other remaining kebeles in the woreda. As indicated in the Table 2, there are more than 9845 donkey population in the three kebeles in which 3920, 2144 and 1697 are found in Gara boru Babogaya and Hiddi respectively (Adea woreda agricultural and rural development office, 2008). The report from Adea woreda agricultural and rural development office (2008) indicates that majority of the donkeys are male donkeys. The livelihood of the population of the woreda is mainly based on crop production and livestock as the 2nd means of livelihood (priority area). The major crops produced in this area include 'Teff,' wheat and highland pulses such as pea, feba-bean and chick pea. Donkey is one of the means of transporting the crop from the field to the home and market areas. This implies that donkey has an importance for the livelihood of the peasant in this area.

Table 2. Human, Livestock and donkey population in Garaboru. Babgaya and Hiddi

Area	Human population			Livestock population					
	M	F	Total	Cattle	Sheep	Goat	Donkey	Horse	Mule
Garaaboru	2587	2152	4739	6688	739	220	3920	2	3
Babogaya	3117	2958	6075	3768	837	715	2144	117	23
Hiddi	3705	3081	6786	6921	987	418	1697	152	37

Source: Report from Adea woreda agricultural and rural development office (2008).

There are four types of donkeys in Ethiopia namely Abyssinia, Ogaden, Jimma and Senar donkeys. Among these types, Abyssinia donkeys were selected for this research purposively. To this end, Adea woreda was selected since large population of Abyssinian donkeys are found in this area. Thirty donkeys, (parity from one to five), selected from the above mentioned areas were used in this study. A preliminary examination was conducted by using 0.1 M sodium hydroxide solution to ensure that the experimental donkeys are free from mastitis.

3.2. Sample collection

Samples were collected from 30 Jennies which are found in different kebeles of Adea woreda. From each jenny, approximately 500 ml of the milk was taken to represent content of the whole population. The collection and transportation of the milk samples were synchronized. The milk was collected on the same day and transported to both laboratories namely micro-biological and food technology laboratories. The reliability of data regarding the content of any component depends highly on correct sampling, transportation, storage and adequate analytical methods in order to minimize the sources of error. Glasses used for the milk samples were acid washed to minimize impurities consisting of trace elements especially for mineral analysis. In addition pH also was checked at the time of arrival at food Engineering laboratory.

Samples were milked manually from apparently healthy Jennies and sampling was continued until the desired sample size was obtained. Samples were collected through direct milking into a sterile test tube/bottle from washed and unwashed udder, for nutritional and microbiological analysis respectively, and transported to laboratories within 3-4 hours using an ice box to maintain at 4 °C.

3.2.1. Sample preparation

The samples were then thoroughly mixed, sub-sampled and samples for the analysis of ascorbic acid and vitamin E were stored in brown bottle. Consequently, samples were transferred to a deep freezer and stored at -20 °C until nutritional analysis was conducted. Microbiological and physical analyses were carried out immediately at arrival in the Drug Research Department of Ethiopian Health and Nutrition Institution, microbiological and Food Engineering laboratories, respectively.

3.4. Instrument and methodology

The parameters and the analytical methods are given in Table 3 below. The parameters analyzed were the main components of milk such as total protein, total fat, lactose, as well as other components of significance from nutritional and microbiological point of view. Components were analyzed from all 24 Jennies (24 samples in total). The methods of analyses followed were that of the Association of Official Analytical Chemists (AOAC) and reference methods of the International Dairy Federation (IDF) and other published methods.

Table 3. Analysis methods used for the quantification of components of donkey milk

Proximate analysis	Methods of analysis
Fat	Gerber method
Protein	Kjeldahl methods
Lactose	Lactoscan method
Vitamin C	Spectrophotometric method
Calcium	Standard method of AOAC (1998)
Magnesium	Standard method of AOAC (1998)
Zinc	Standard method of AOAC (1998)
Iron	Standard method of AOAC (1998)
Phosphors	Spectrophotometric method (1998)
PH	pH meter
Total solid	IDF-ISO-AOAC method (1999)
specific gravity	pycnometric method
Acidity	Titrimetric method (AOAC, 1947)
Freezing point	Lactoscan method
Isolation and Identification of microbes	International standard ISO 8261.

3.4.1. Physical analysis

PH was determined by using a digital pH meter (pH test 10 DOUBLE JUNCTION Eutech instrument), and density was determined at 15°C by pycnometric method (AOAC, 1999). Total solids were determined gravimetrically after the milk was dried in a forced-convection oven at 105°C until a constant weight was achieved according to IDF-ISO-AOAC method (AOAC,

1999), while acidity was determined by titrimetric method (AOAC, 1947). Acidity is reported as % lactic acid by weight in which 1 ml of 0.1M NaOH = 0.0090 g lactic acid.

3.2.2.2. Proximate analysis

Fat content was determined by using Gerger-method. Prior to analysis, each sample was warmed to 38°C in water bath to disperse the cream and reincorporate any cream adhering to container or stopper and mixed until homogenized. Then the warmed test samples were cooled to 20°C. Following this, 10.77 ml of pre-treated samples were poured into butyrometer containing 10 ml of sulfuric acid. One ml of amylalcohol was added into the butyrometre and shaken until the content is thoroughly mixed; i.e, until no white particle of milk could be seen by inverting the butyrometer during the process. The butyrometers were placed into the Gerber which maintain a temperature of 65°C for 5 minutes while centrifuging at speed of 1100 r.p.m. The fat percentage was recorded by observing the fat column.

Nitrogen content of donkey milk was determined by the Kjeldahl method and a nitrogen conversion factor of 6.38 was used to calculate protein contents of donkey milk samples. Donkey milk was digested in H₂SO₄, using CuSO₄·5H₂O as catalyst with K₂SO₄, as boiling point elevator, to release nitrogen from protein and retain nitrogen as ammonium salt. Concentrated NaOH was added to release NH₃, which is collected in H₃BO₃ solution and titrated.

Volume of HCl was recorded and the results were calculated by using the following formula:

$$\text{Nitrogen, \%} = [1.4007 \times (V_s - V_b) \times M] / W$$

Where V_s and V_b = mL HCl titrant used for test portion and blank, respectively; M = molarity of HCl solution; and W = test portion weight, g. Percent nitrogen was multiplied by factor 6.38, to calculate percent "protein" on a total nitrogen basis.

The milk constituent's lactose and physical characteristics such as freezing point of the milk were determined by milk analyzer Lactoscan 90 LCD (Aple Industries services–La Roche Sur Foron, France) based on manufacturer's instructions. Milk samples were mixed gently to avoid any air enclosure in the milk. Then 25 ml samples were poured in to the sample-holder with the

analyzer one at a time and when the analyzer sucks the milk, The measurements, was carried out after the starting button was activated. Finally the digital indicator display shows the specified results.

Vitamin C content of donkey milk was determined using spectrophotometric method. Milk samples were centrifuged at 3000 rpm for 20 minutes. The fat component of milk separates from the fluid at the top, and the cells settle at the bottom. From the clear mid-zone, 0.3 ml of milk was taken and 1.2 ml trichloroacetic acid was added and mixed well. The mixture was then centrifuged at 3000 rpm for 10 minutes. 0.9 ml of the supernatant was taken and 0.4 ml of 2, 4 dinitrophenyl hydrazine thiourea copper sulfate) solution was added. It was then covered with aluminum foil and incubated at 60°C for 60 minutes in a water bath. Then samples were chilled in ice-cold water immediately and 1.6 ml of 65% sulfuric acid was added gradually. Then the treated sample was stored at room temperature for 30 minutes. Absorbance was measured against a reagent blank at 520 nm by a spectrophotometer (UV-v3, Thermo Electrocorporation). Every sample was analyzed twice to obtain duplicate readings (Luthfor *et al.*, 2004)

In preparation of standard, 1000 mg/dL (w/v) ascorbic acid stock standard was made by dissolving 1g of L-ascorbic acid in 30 ml of distilled water in a 100-mL volumetric flask and mixed well and dilute to volume. From this 5 ml was taken and added into a 200-ml volumetric flask and diluted to volume with distilled water to form intermediate standard. Finally in a series of 50-ml volumetric flasks (Elaine, *et al.*,1976-1980) , working standard solution was prepared (Table 4 and Figure 1).

Table 4. Working Vitamin C standard preparation

Standards	ml Intermediate Standard	Actual Cone. [mg/dL] Standard Solution
1	0	0
2	4	2
3	8	4
4	12	6
5	16	8
6	20	10

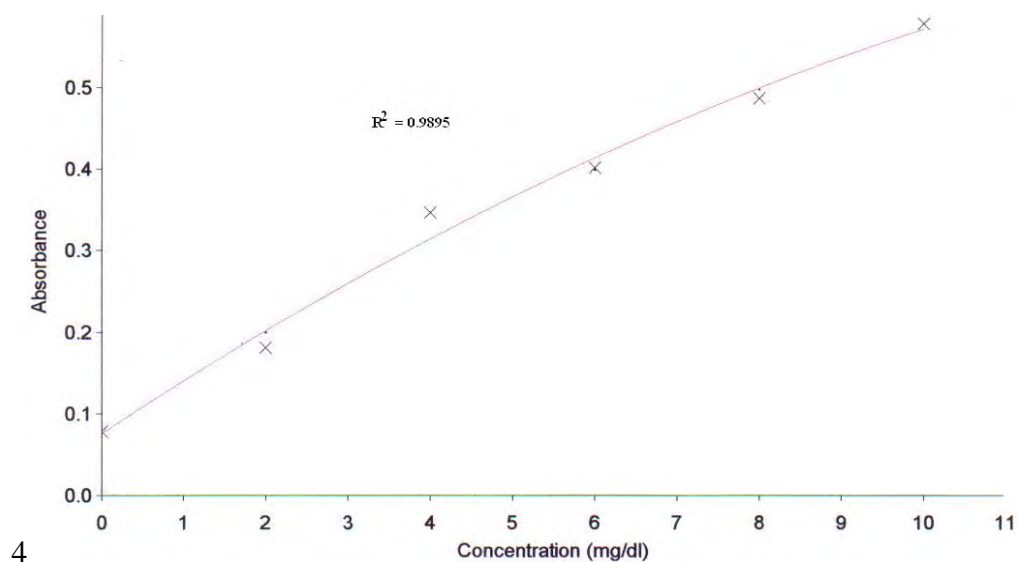


Figure 1: Standard curve for ascorbic acid determination

34.3. Mineral analysis

The analytical determination of the macro elements Ca, Mg, and the micro element Fe and Zn were performed using an Atomic Absorption Spectrometer (Varian, Spectra 220). After removal of organic matter by wet digestion, liquate was diluted with water according to standard Association of Official Analytical Chemistry method (1998). On the other hand, phosphorous was determined by spectrophotometer method from wet digestion test portion by complexing with ammonium molybdovanadate reagent. Twenty milliliter of molybovanadate and 20 ml of

pre-prepared test solution was added to 100ml of volumetric flask and diluted to the mark with distilled water. After 10 minutes, phosphorous was determined in the form of phosphate at 400 °C.

The inorganic acids (concentrated HNO₃ and H₂SO₄) used were all of analytical grade and a materials and glasses were thoroughly cleaned with HNO₃ to 10% v/v. Samples were prepared in a very clean place under clean conditions in Ethiopian standard and quality authority laboratory. The methodology used, consisted of introducing 5.0 mL sample of milk which was weighed and placed in Kjeldahl flask (capacity of 100 mL). Following, 10.0 mL of concentrated H₂SO₄ and 5.0 mL of concentrated HNO₃ were added. After that, it was cautiously heated until the initial vigorous reaction has subsided. Heating was continued until most of the nitrous fumes are removed. Then HNO₃ was added drop wise until all organic matter is destroyed and heated until white fumes of sulfuric acid was evolved and solution become colorless. Blank was prepared using the same amounts of reagents as sample. Solutions obtained from wet digestion was transferred from the flask to a graduated volumetric flask and filled up to mark with water and mixed thoroughly. Finally, measurements of the elements content were carried out following the instruction given for the relevant metal.

The apparatus was set according to the instructions of the instrument. The standard and the reagent blank solution were measured and calibration curve was prepared by plotting the absorption values against the metal concentration in mg/L. This task was performed automatically by atomic absorption by providing only one solution of known concentration of each element for Ca, Mg, Fe and Zn, unlike phosphorous which involves preparation of series of working standard solution of 0.0, 5.0, 8, 10 and 15 (Table 5 and Figure) for plotting calibration curve on spectrophotometer. Then P was determined by using the following formula:

$$P = 100 * V_2 * p / V_1 / W$$

Where: W= Weight of samples

V₁= Volume of dilution

V₂= Volume molybovanadate solution

p = Concentration [mg/l] phosphate ion

P = Phosphorus content (mg/kg)

Table 5. Working standard for Phosphorus determination

Standards	Actual Con. [mg/l] Standard Solution
1	0
2	5
3	8
4	10
5	15

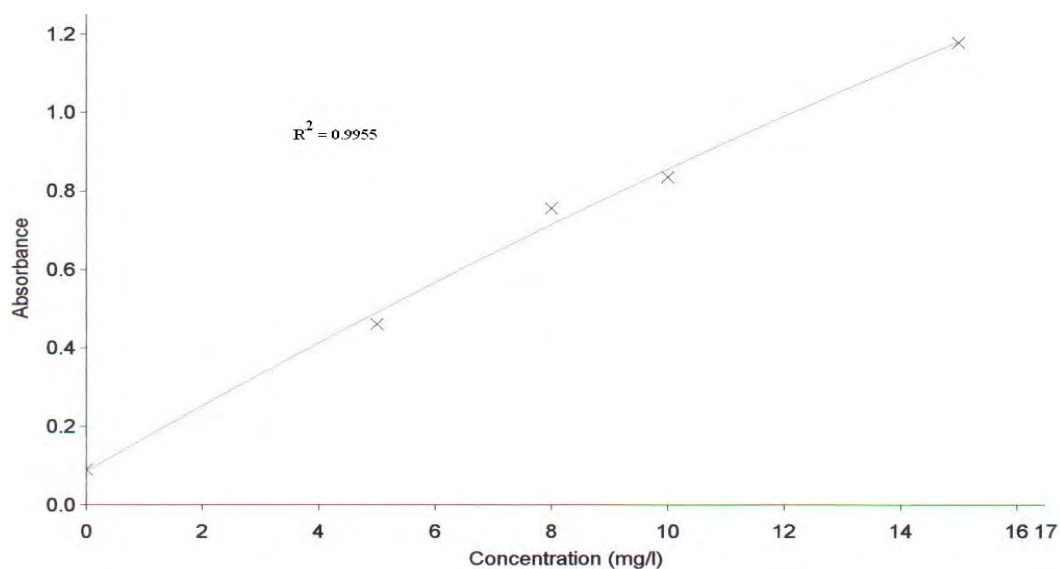


Figure 2: Standard curve for phosphorus determination

The concentration [mg/l] of specific metal (Ca, Mg, Fe and Zn) that correspond to the absorption values of the samples and blank were read from the graph and calculated.

$$\text{Metal content (mg/kg)} = [(a-b) \times V]/$$

Where: W= Weight (g) of samples

V= Volume (V) of dilution

a = Concentration ($\mu\text{g/ml}$) of sample solution

b = Concentration ($\mu\text{g/ml}$) of blank solution

3.4.4. Microbiological analysis

This study was conducted in Addis Ababa at Ethiopian Health and Nutrition Research Institute (EHNRI) at the department of Drug research and Institute of Biodiversity. A minimum of 45 to 50 isolates were isolated from each sample on different media at different temperature of incubation, most of which later on are reclassified in to few groups on preliminary biochemical test. These samples were again re-grouped into three main groups each of which has its own 4 sub-groups. First batch of samples are raw donkey milk samples collected from individual farmer's homes located at about 10 km on the southern direction of Adea town from farmer association specifically known as Gara Boru. These samples were used to monitor changes in microbial spectrum and population kinetic of raw as well as fermented (further incubated) donkey milk samples. Second batch of samples are raw donkey milk samples collected from individual farmers homes located at about 5 km on the southern direction of Adea town from Babogaya area as mentioned for Firs Batch Sample. Third batch of samples are raw donkey milk samples collected from individual farmers homes located at about 20 km on the southern east of Adea town called Hiddi area for the same analysis as mentioned under first batch and second batch of samples. The samples were immediately transported to the laboratory for zero-hour sampling. In each case samples were collected, in pre-sterilized bottles with a holding capacity of 30 ml. Then fermentation was initiated in these bottles with the left over samples after portions of samples are taken for zero hours sampling.

Fermentation process was allowed to proceed for about 18 days at room temperature as it was not easy for donkey milk to ferment within this time of incubation unlike cow milk. Aliquot (10 ml) of the milk sample was taken for each bacteriological analysis after homogenization of each sample was done under aseptic conditions. Both general as well as selective media were used for isolation of various groups of microbial colonies. PCA (plat count Agar), PDA (potato Dextrose Agar), M17 (M17- Agar), MRS (Mann Rogosa Sharpe Agar), and MacConkey were used to cultivate and isolate Total Aerobic bacteria (TAB), Fungi (Yeast and Mould), G.P (gram positive), and gram negative groups respectively. To grow and isolate each expected organism, pour plate method was used for all media listed above; i.e., to each plate 15 to 20 ml of molten agar at 45⁰c to 50⁰c was added. Then 1 ml of inoculums from each dilution is required into each

Petri-dish. The medium in each plate and the inoculums was mixed by shaking and circular movements lasting 8 sec to 1 minute. All plates were allowed to set until solidified.

Each plate was then inverted and incubated at appropriate temperature. All the plates are labeled fully (i.e. the label contained at least name of the media, dilution factor, temperature of incubation, code of the sample, time of the experiment). All counts were determined by serially diluting the milk samples 10 fold. In this case, after the milk samples were homogenized, about 10 ml of the sample was added to 90 ml of buffered peptone water to prepare the first 10 fold dilution in each case then this was further diluted up to 10^6 using again the same buffered peptone water. One ml was inoculated in duplicate from each dilution on each medium to obtain countable colonies in each plate.

Incubation was carried out at 30°C for M17 and MRS plates (gram positive) and at 37°C for PCA (Total Aerobic Bacteria) and MacConkey (gram negative group) plates, While it was at 25°C for PDA (Yeast and Mould) plates. The incubation periods were 24 to 72 hrs for all. But sometimes when there is no good growth the incubation period was extended up to over 1 week. Plates having between 30 to 100 colonies were counted and colony forming units per milli litter (cfu/ml) of test samples were calculated.

The major of the preliminary biochemical tests were carried to group them at least into genus and group level for isolates of each sample. Some of the main preliminary biochemical tastes are like, Catalase test, Oxidase test, morphology (using Phase Contrast Microscopy technique). Cultural Characters and morphology were the primary methods used as the first step, in identification & characterization of the colonies for further biochemical tests. Purification of each colony for further biochemical tests to classify each colony (isolate) to at least genes level was done on PCA plates i.e. each isolate was re-streaked on solidified PCA plate to be checked for purity before running any biochemical test. Finally each pure organism was transferred in to slant in their respective media and stored at 4°C .

Identification and characterization of gram positive microorganism

Identification and characterizing of microorganisms were performed following procedure given by OmniLog Identification manual. Before the identification, a pure culture was isolated on OmniLog Bug Agar and Gram Stain was done. The inoculums cell density was prepared using turbidometer which was set according to the Gram staining classification. The prepared inoculums were introduced in a labeled microplate and were inoculated in a strictly aseptic condition. The microplates were placed in OmniLog Plus ID Chambers after necessary data were loaded to ID system. The OmniLog ID provided reading of results and hard copy prints t after an ID was completed (Biolog inc., 2006)

3.5. Statistical Analysis

Data were analyzed by SPSS program (Statistical Package for Social Science, version 15.00. 2007). This test combines ANOVA with comparison of differences between means of the location.

Chapter – 4

RESULTS AND DISCUSSION

4.1. Physicochemical analysis

Physical characteristics of milk (such as pH, density, titratable acidity, total solids) are important parameters in studying its physicochemical aspects. Table 6 shows the various physical parameters of the Abyssinian donkey milk samples.

Table 6: physical properties of Abyssinian donkey milk samples collected from the study sites

Parameter	N	Mean \pm SD	CV	Minimum	Maximum
pH	24	7.20 \pm 0.12	0.016	7.0	7.40
Density [g/cm³]	24	1.026 \pm 0.006	0.0058	1.01	1.04
Acidity [%]	24	0.14 \pm 0.15	1.07	0.04	0.53
TSS [%]	24	8.80 \pm 0.98	0.11	6.56	12.16
F point [⁰C]	24	-0.45 \pm 0.065	-0.144	-0.56	-0.27

PH is the parameter that determines the sample acidity and alkalinity. The mean level of pH of donkey milk was found to be 7.2 \pm 0.12. The coefficient of variation in the same table (0.016) shows that the pH level did not vary among individual donkeys. Compared with this statistic, Guo and colleagues (2007) found out a pH level of 7.18 \pm 0.13 for donkey milk and 6.89 \pm 0.08, for human milk (Table 1). Salimei *et al.*, (2004) indicated that the pH level for donkey milk was in the range of 7.14 to 7.22. Comparison of the pH value of the present study with the literature value for donkey milk suggested that there is no statistically significant difference between the two ($t_{92} = 0.66$, $P > 0.01$, Table 9) while the difference is significant with the human milk ($t_{42} = 8.61$, $P < 0.01$, Table 9). This suggests that the pH value may not be influenced by possible breed variation for donkeys. The average pH value (7.2) of donkey milk was higher than cow milk pH ranges from 6.3 to 6.8 (Van den Berg, 2006) which is slightly acidic.

Milk density is another important parameter. It is influenced by factors like inclusion of air during delivery, adulteration of water and skimming. So, the density measurement of milk is used for quality control. Density of donkey milk in this investigation has the mean value of 1.026 \pm 0.006. This value is similar with literature (Guo, *et al.*, 20007) for both donkey (1.03 \pm 0.02,) and human milk 1.027 \pm 0.011 (Table 1) because, the mean comparison of the present donkey milk

with literature donkey milk data ($t_{172} = -0.968$, $P > 0.01$, Table 9) and also with human milk ($t_{42} = 1.30$, $P > 0.01$) presented on Table 9 shows that there is in significant difference between the means.

As an indicator of milk quality, milk acidity is used to measure and monitor such processes as making cheese and yogurt. Accordingly, due to the buffering capacity of protein and milk salts, fresh cow milk exhibits an initial acidity of 0.14 to 0.16 (O'Connor, 1995) when titrated with 0.1 NaOH N. In this study, the acidity value obtained for donkey milk was in the range of 0.53 to 0.14 which is lower than cow milk acidity. When Acidity of donkey milk investigated in this study was compared with human milk (0.072 ± 0.011 , Table 1) Elisabetta and colleague (2009), no statistically significant difference was found between the two means ($t_{172} = 1.98$, $P > 0.01$, Table 9).

Finally, the dry matter observed in current donkey milk study (average of 8.8 ± 0.98 %) was also compared with the one in the literature (Chiavari, 2005) which reported a mean average of (8.91 ± 0.31 , for donkey and 10.71 ± 0.11 (Table 1) for human milk. The mean test shows that there is a significant difference between the two mean ($t_{42} = 8.47$, $P < 0.01$), while the dry matter content of the current donkey milk is consistent with the donkey milk content in literature ($t_{172} = -1.07$, $P > 0.01$ Table 9). The means of freezing point of donkey milk samples studied was -0.45 °C. The result obtained in this study is greater than freezing point for cow milk as investigated by Samia *et al.*, (2009).

4.2. Proximate analysis

Protein, Fat and Lactose are among the basic group of milk component. The Abyssinian donkey milk samples were also subjected to the determination of the amount of total protein, fat, lactose, vitamin C as presented on Table 7. Minimum and maximum values, overall means of gross composition, standard deviation, and coefficients of variation are reported in this Table.

Table 7. Composition of Abyssinian donkey milk samples collected from Garaboru, Babogaya and Hiddi area

Item	N	Mean (\pm SD)	CV	Minimum	Maximum
Lactose [%]	24	6.29 \pm 0.45	0.07	5.39	7.01
Fat [%]	24	0.36 \pm 0.11	0.30	0.23	0.67
Protein [%]	24	1.98 \pm 0.40	0.17	1.43	2.89
Vitamin C [mg/dl]	24	3.15 \pm 1.18	0.37	1.25	6.23

The observed average protein content of the present donkey milk (1.98) was also compared with the one in literature for donkeys (1.56 \pm 0.14, Table 1) and humans (1.94 \pm 0.14, Table 1). Results revealed that it was consistent with Elisabetta *et al.*, (2009) data for human milk ($t_{42}=0.048$, $P>0.01$, Table 9) but significantly different from its own species ($t_{42}=8.38$, $P<0.01$, Table 9).

Fat is the most variable component in milk both in its concentration and chemical composition, despite species differences. Milk fat is the major source of lipid used by the mammalian newborn for accumulating body adipose tissue. The data in this study revealed that the average fat content of donkey's milk investigated in this study indicated in Table 7 was 0.36% \pm 0.11 and similar with the values reported by Salimei *et al.*, (2004) for donkey (0.38% \pm 0.12, $t_{92} = 0.36$, $P>0.01$,) but lower than Elisabetta and colleague' (2009) reported for human milk (2.1% \pm 0.15, $t_{42} = 43.25$, $P<0.01$, Table 9).

Lactose is a component of milk considered. It is unique to milk (i.e. does not exist anywhere else in nature) and it plays a major role in milk synthesis. Because of the close relationship between lactose synthesis and the amount of water drawn into milk, lactose is the least variable component of milk. Lactose is the major milk carbohydrate in most species. Some species have very little lactose in milk (e.g. bear and kangaroo), while others (e.g. donkey, human) have high as 7% lactose in their milk. Lactose is important in the manufacturing of many fermented dairy products such as yogurt (O'Connor, 1995). Considering our present data summarized in Table 7, the high lactose content determined in this study (mean of 6.29 \pm 0.45) was consistent with Guo *et al.*, (2007) reported data for donkeys (mean 6.33 \pm 0.17, $t_{92}=-0.62$, $P>0.01$), and humans (mean

6.45± 0.08, $t_{42}=-0.7$ P>0.01). Finally, the mean vitamin C content in the present donkeys (3.15 ± 1.18) was compared with human milk in literature (3.06 ±0 .67) (Luthfor *et al.*, 2004). And test results on Table 9 yielded that there is no significant difference between the two.

4.3. Mineral Analysis

Table 8 summarizes the result analysis for Ca, Mg, Zn, Fe, and P contents in Abyssinian donkey milk samples collected from the study sites

Table 8. Mean (\pm SD) of mineral composition of Abyssinian donkey milk samples collected from Garaboru, Babogaya and Hiddi are

Milk Contents	N	Mean	CV	Minimum	Maximum
Calcium [mg/kg]	24	737.46 ± 309.85	0.42	435.73	1895.32
Magnesium [mg/kg]	24	58.71 ± 22.40	0.38	30.11	137.13
Iron [mg/kg]	24	24.06 ±28.05	1.17	3.54	94.73
Zinc [mg/kg]	24	5.56 ± 3.38	0.61	1.68	14.84
Phosphors [mg/kg]	21	314.99 ± 151.69	0.48	87.25	0.63

Mean Ca content of donkey’s milk was 737.46 ± 309.85 mg/kg, almost closer to Salimei *et al.*, (2004) reported value for donkey milk (mean, 677 ± 282.85 mg/kg, $t_{46} = 0.69$, P>0.1). But one fold higher when compared to a previous study by Elisabetta *et al.*, (2009) for donkey milk (334.61 and human milk (340.00 mg/kg, $t_{42} = 5.87$, P<0.01). There is significance difference between what was investigated by this study and previous report by Elisabetta *et al.*, (2009). Moreover, the observed Ca content was higher than that reported for cow’s milk (680 ± 79.8 mg/k) or other milk from ruminants such as goat (646 ±76.6 mg/kg) Elisabetta *et al.*, (2009).

Mean Mg content of donkey’s milk (58.71± 22.4 mg/kg) is 2-3 times lower than from the one reported (Mohammad, *et al.*, 2008) for goat (139.15.7 mg/kg) and bovine milk (205.22.1 mg/kg) but almost similar with that reported in the literature for donkey milk (58. 46 ± 8.43 mg/ kg) by Elisabetta *et al.*, (2009) ($t_{46}= 0.14$, P>0.01). The Mg content observed in the present study was, however, significantly higher than what was previously observed in human milk (30± 0.35

mg/kg) by Elisabetta and *et al.*, (2009) ($t_{42}=79.31$, $P<0.01$). The same can be said with the Mg content in human milk previously reported to range from 15 to 64 mg/L with a median value of 31 mg/L (Dorea, 2000). Mean Zn content of donkey's milk obtained in this study (5.56 ± 3.3 mg/kg) was significantly different from what was observed in human milk (2.1 mg/kg, $t_{42}= 4.2$, $P>0.01$) and donkeys' milk (1.22 ± 0.52 mg/kg, $t_{46}= 6.1$, $P>.01$). It was demonstrated that Zn in human milk is more efficiently utilized compared to bovine milk because of the high casein content may be the same is true for donkey milk due to close similarity of chemical composition.

Table 9. Test of differences between contents of Abyssinian donkey milk in the present study and literature values for human milk and donkey milk

Parameters	Abyssinian and literature donkey milk		Abyssinian and literature human milk	
	df	t _{calculated}	df	t _{calculated}
pH	92	0.66	42	8.61
Density	172	-0.968	42	1.30
Acidity	-	-	42	1.98
TSS	172	-1.07	42	8.47
Fat	92	0.36	42	43.25
Protein	92	8.38	42	0.048
Lactose	92	-0.62	42	-1.54
Vitamin C	-	-	48	0.33
Calcium	46	0.69	42	5.87
Magnesium	46	0.14	42	79.31
Iron	46	3.92*	42	3.69*
Zinc	46	6.10	42	4.20
Phosphorus	-	-	42	4.46

* All values are significant at $p<0.001$

The mean Fe content of donkey's milk (24.06 ± 28.05 mg /kg) was much higher than those observed by Elisabetta and *et al.*, (2009) for human (0.4 ± 0.0 mg/kg, $t_{42}=3.69$, $P<0.01$) and donkey milk (1.15 ± 0.51 mg/kg, $t_{46}= 3.92$, $P<0.01$). Fe content of donkey' milk showed a high variability, ranging from 3.54 to 94.74 mg/kg. In the present study, the mean content of P in donkeys' milk (314.99 ± 151.7 mg/kg) was higher than human milk (160 ± 0.85 mg/kg, $t_{42}= 4.46$, $P<.01$) (Elisabetta *et al.*, 2009).

4.4. Factors affecting donkey milk composition

Attempts were also made to determine if milk composition of the sampled donkeys could differ location-wise, and along with the lactation period. One-way ANOVA was conducted to test location effect (Table 10).

Table 10: Differences in physicochemical parameters and composition of Abyssinian donkey milk contents among the three study sites

Parameters	Mean Square	F	P-value
pH	0.106	16.440	0.000
Density	0.000	8.555	0.002
Acidity	0.010	0.444	0.647
TSS	0.485	0.482	0.624
Freezing point	0.006	1.493	0.248
Protein	0.97	0.907	0.576
Fat	0.024	2.179	0.138
Lactose	0.196	1.081	0.357
Vitamin C	0.481	0.324	0.727
Calcium	61509.011	0.619	0.548
Magnesium	969.364	2.19	0.145
Iron	2777.912	4.813	0.020
Zinc	42.524	4.726	0.020
Phosphorus	29023.820	1.299	0.297

As indicated in Table 10, it is only pH ($F_{2, 21} = 16.44$, $P < 0.000$) and density ($F_{2, 21} = 8.56$, $P < 0.002$) of the sampled donkey milk compositions that were found to significantly differ location-wise. The absence of difference is probably due to their similarity in their feeding system which is based on grazing and similarity in agro ecological condition.

4.5. Microbiological analysis

Table 11. Mean value of microbial count for TAC, GN bacteria, G.P positive, Yeast and mold in log CFU/g of donkey milk Samples collected from the three sampling sites

Location	Time of incubation	Expected microbial group				
		TAC [log cfu/ml]	Gram negative [log cfu/ml]	Gram positive [log cfu/ml]	Yeast [log cfu/ml]	Mold [log cfu/ml]
GaraBoru	0 hr	5.66	5.40	2.32	1.72	2.45
	18 days	6.32	4.81	3.38	4.69	1.40
BaboGaya	0 hr	4.62	5.01	3.08	1.18	1.25
	18 days	6.00	4.48	6.07	1.00	1.14
Hiddi	0 hr	3.99	3.11	3.15	2.72	2.30
	18 days	6.2	2.77	6.74	1.48	1.00
Mean total	0 hr	4.75	4.5	2.85	1.87	2.00
	18 days	6.17	4.02	4.75	2.39	1.18

The mean average of three sampling site initial donkey milk contained of mean 4.75 log cfu/ml of total count bacteria (TAC), gram negative, 4.5 cfu/ml, Gram positive 2.85 cfu/ml, 1.87 log cfu/ml of yeast and 2.00 log cfu/ml of mold (Table 11). At room temperature, TAC and gram positive bacteria increased progressively during storage for 18 days to the value of 6.17 and 4.75 log cfu/ml. While gram negative, yeast and mold reached the counts of 4.02 log cfu/ml, 2.39 log cfu/ml and 1.18 log cfu/ml respectively, under the same conditions. Initial raw donkey milk contained a low microbial content with a mean total count (TAC) of 4.75 log cfu/ml, while the loads of microbe of bovine milk were approximately 7 cfu/ml, (Chye, Abdullah, & Ayob, 2004; Morgan et al., 2003). The reason for the low microbial counts in donkey milk is likely due to the natural antimicrobial substances present in it.

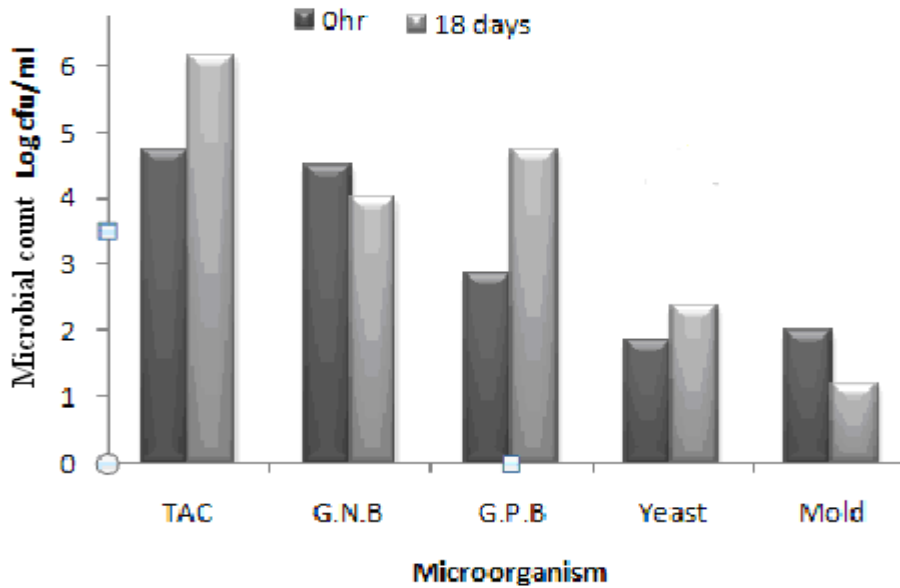


Figure 3. Change in microbial flora of Abyssinian donkey milk samples during storage for 18 days

During microbiological investigation Gram negative, Gram positive, aerobic bacteria, yeasts and moulds were isolated (Table 11). The isolates were initially differentiated on the basis of their cultural and morphological studies after which they were subjected to Catalase and Oxidase tests. A total of 150 colonies morphologically observed. Morphological identification of the isolates led to species identification of those gram positive bacterial isolate. From this 20 bacterial isolates suspected as lactic acid bacteria were used for further species identification by OmniLog Puls Identification System at the Microbial laboratory of the Institute of Biodiversity Conservation (Table 12).

Table 12. Bacterial strains isolated from Abyssinia donkey milk that were suspected as lactic acid

Strain Code	Microbial flora count [cfu/ML]		Identification	
	0 hrs	18 days	Morphological	OmniLog Plus System ID
DM-11	3x10 ²	2x10 ³	Cocci (mostly diplo cocci)	-
DM-29	8x10 ¹	-	Rod (short)	<i>Clavibacter agropyri</i> (corynebacterium)
DM-31	6.2x10 ²	2x10 ³	Rod (short)	-
DM-33	2x10 ²	1x10 ³	Rod (very short)	-
DM-34	1x10 ²	4x10 ³	Rod (very short)	-
DM-69	3x10 ²	4x10 ⁵	Cocci (mostly diplo cocci)	-
DM-77	3x10 ³	3.52x10 ⁶	Cocci (mostly diplo cocci)	-
DM-85	4x10 ²	8x10 ⁴	Rod (short to medium)	-
DM-86	6x10 ²	1.6x10 ⁵	Rod (short)	-
DM-91	2x10 ²	7.2x10 ⁴	Cocci (diplo + short chain)	-
DM-92	3x10 ²	1.28x10 ⁴	Cocci (dispersed + chain forming)	-
DM-99	1.6x10 ³	4x10 ⁶	Cocci (dispersed + chain forming)	-
DM-105	2x10 ²	4.8x10 ⁶	Cocci (diplo + chain forming)	-
DM-106	5x10 ²	-	Cocci (mostly diplo + short chain)	-
DM-107	3x10 ²	2.8x10 ⁶	Cocci (diplo + short chain)	-
DM-120a	1x10 ²	-	Rod (medium size –thin)	<i>Clavibacter agropyri</i> (corynebacterium)
DM-135	3x10 ²	1x10 ²	Cocci (single + diplo + tetra)	-
DM-136	2x10 ³	2.2x10 ⁶	Cocci (slightly bigger, diplo + short chain)	-
DM-149	2x10 ²	-	Rod (very short)	-
DM-150	4x10 ²	-	Rod (single + short chain)	<i>Leifsonia aquaicat</i>

Only three isolates (Sample No. 29, 120a and 150) have showed successful grown on the OmniLog Plus ID System BUG Agar which is a prerequisite growth medium for identification System. No generous growth of all isolates was observed during one week incubation at 30⁰C to obtain enough culture for OmniLog Plus ID System analysis. According to the OmniLog Plus Identification, two different species, i.e. *Clavibacter agropyri* (*Corynebacterium*) for isolates No. 29 and 120a; and *Leifsonia aquatica* for isolate No 150 were identified with Low similarity. It was not possible to conclude the presence or absence of lactic acid bacteria by OmniLog Plus ID System in this study, since all of organisms that were suspected as lactic acid, were not grown on Bug agar. It could be research gap for others.

Generally, the overall fermentation trend and the total microbial spectrum of donkey milk samples are observed to be different from other dairy milk samples. In the first place, the distribution of bacteria resembles LAB was very low both in number and in kind in all analyzed samples as compared to the non-LAB groups and secondly, the role of this bacteria was also very insignificant unlike in the case of other dairy products fermentation in which they are known to play a very significant role. Thus in the current study, the organism resembles lactic acid bacteria were not capable of fermenting donkey milk, this indicates there are no fomenters bacteria, or most likely due to the effect of antimicrobial factor in donkey milk. Donkey milk does not have the normal fermentation trend but it showed separation of solid at the bottom and liquid phase at the top. It was incubated for 18 days to observe fermentation trend. During this incubation time pH level showed decreasing values. The pH of the donkey milk samples remained constant after 4.8 pH level for the entire time of the trials. In this case the microbial loads of some microorganism such as organisms seems lactic acid bacteria were increased while others such as gram negative and mold were decreased. Generally samples of donkey milk showed low microbial counts. The overall low microbial count may be linked to the high content of lysozyme, one of the milk components with is useful in bactericidal properties.

Chapter – 5

CONCLUSION AND RECOMENDATIONS

In this study, donkey milk resembles with human milk in basic composition of milk such as protein, lactose, vitamin C and physico-chemical characteristic such as density and acidity. But higher value in minerals composition was observed. The current result obtained for Abyssinia donkey milk closely resembles with existing literature value for donkey milk all most in all investigated parameters except Iron Unlike a previous assumption and as it occur in the cow's milk, donkey milk doesn't ferment under natural condition, but there is a drop in pH due to the action of microorganism. Samples of raw donkey milk also showed a low microbial counts further confirming its potential use as safe natural food for infant feeding. Moreover, most of the microorganisms that seem lactic acid bacteria during the isolation did not grow on MRS, M-17 and Bug agar, during OmniLog identification. This research has provided some data that donkey milk can somehow fill in the gap when breast milk can't be given to infant. But, before buying this recommendation further research need to be conducted in donkey milk sensory property, failure of lactic acid bacteria to grow on MRS, M-17 and Bug agar. Further investigation is also needed to know whether donkey milk can suppress the growth of potential pathogens. Based on the above conclusions the following points are recommended:

- More researches are needed on wider scale to characterize donkey milk properties.
- In the study area, the average milk yield of the morning milking of donkeys was found to be very low compared to cow's milk. Establishment of donkey-breeding centers has to be encouraged to develop, and select donkey breeds to improve milk production, and commercialize donkey milk.

Chapter – 6

REFERNCES

REFERENCE

- Adea woreda Livestock development and Marketing office (2000). Annual Report on Livestock development.
- Ahmed, M.M., Assegid, W., Jabbar, M.A., Ayele, S. and Belachew, H. (2003). Livestock marketing in Ethiopia: review of structure, performance and development initiatives. Socio-economic and policy Research Working paper 52.ILRI,Nairobi, Kenya.35pp.
- Alberse, R.C., Stapel, O., (2001). Structure of food allergens in relation to allergenicity: Pediatric. Allergy Immunol. 12 (Suppl. 14), 10–14.
- AOAC, (1980). Official Methods of Analysis 13th ed: Association of Official Analytical Chemists, Washington, DC.
- Bahna, S.L., Gandhi, M.D. (1983b). Milk hypersensitivity: II. Practical 295–301.aspects of diagnosis, treatment and prevention. Ann. Allergy 50.
- Biolog, inc. (2006). Omnilog Data Collection Software Identification System User Guide: Hayward, USA www.biolog.com
- Blench, R.M and MacDonald K (2000). The origins and development of African livestock archaeology, genetics, linguistics and ethnography: University College London Press, London, UK. ISBN 1-84142-018.
- Burns, J.P. (2001). HIV, Breastfeeding, and Infant Mortality: Weighing the Impact of the Non-evidenced-based WHO/UN AIDS. Recommendations in the Low-income, Low-resource Countries of Sub-Saharan Africa. Boston University, School of Public Health Department of International Health.
- Carroccio, A., Cavataio, F., Iacono, G. (1999). Cross-reactivity between milk proteins milk proteins of different animals: Clin. Exp. Allergy 29:1014-1016.
- Carroccio, A., Cavataio, F., Montaldo, G.D., Amico, D., Alabrese, L., and Iacono, G. (2000). Intolerance to hydrolyzed cow's milk protein in infants: Clinical characteristics and dietary treatment. Clin.Exp. Allergy 30:1597–1603.
- Central Statistical Authority, (2006). Ethiopia Demographic and Health Survey: Addis Ababa, Ethiopia.
- Central Statistical Authority, (2008). Statistical Abstract: CSA, Addis Ababa/ Ethiopia.

- Chandan, R.C., Parry, R.M., and Shahani, K.M. (1965). Lysozyme, lipase and ribonuclease in milk of various species: *Journal of Dairy Research*, 51, 606–607.
- Chiavari, C., Coloretti, F., Nanni, M., Sorrentino, E., and Grazia, L. (2005). Use of donkey's milk for a fermented beverage with lactobacilli: *Lait*, 85, 481–490.
- Chihara, G. (1992). Immunopharmacology of lentinan, a polysaccharide isolated from *Lentinus edodes*: its application as a host defense potentiator. *International Journal of Oriental Medicine*, 17, 57–77.
- Chye, F. Y., Abdullah, A., & Ayob, M. K. (2004). Bacteriological quality and safety of raw milk in Malaysia: *Food Microbiology*, 21, 535–541.
- Coppola, R., Salimei, E., Succi, M., Sorrentino, E., Nanni, M., Ranieri, P. (2002). Behavior of *Lactobacillus rhamnosus* strains in ass's milk: *Annals of Microbiology*, 52, 55–60.
- D'Alessandro, A.G., Martemucci, G. (2007). Influence of milking number and frequency on milk production in Martina Franca breed asses.
- Donnet-Hughes, A., Duc, N., Serrant, P., Vidal, K., Schiffrin, E.J., (2000). Bioactive molecules in milk and their role in health and disease: The role of transforming growth factor- β . *Immunol. Cell Biol.* 78:74-79.
- Doreau, M., Boulot, G. (1989). Methods of measurement of milk yield and composition in nursing mares: A review. *Lait* 69, 159-171.
- Dorea, J.G. (2000). Magnesium in human milk: *J. Am. Coll. Nutr.* 19. 210-219.
- Dugo, P., Kumm, T., Lo Presti, M., Chiofalo, B., Salimei, E., Fazio, A., Cotroneo, A., Mondello, L. (2005). Determination of triacylglycerols in donkey milk by using high performance liquid chromatography coupled with atmospheric pressure chemical ionization mass spectrometry: *J. Sep. Sci.* 28:1023-1030.
- Elaine W, Gunter, Wayman E., Turne Jane W., Neese, Ph.D., and David D., Bayse, Ph.D., (1976-1980). Laboratory Procedures used by the Clinical Chemistry Division, Centers for Disease Control, for the Second Health and Nutrition Examination Survey.
- El-Agamy, E.I. (2006c). Camel milk proteins are suitable for the nutrition of cow's milk protein allergic patients: *J. Food Chem.*, in press.
- El-Agamy, E.I., Abou-Shloue, Z.I., Abdel-Kader, Y.I. (1997). A comparative study of milk proteins from different species: II. Electrophoretic patterns, molecular characterization, amino acid composition and immunological relationships.

- Elisabetta S., Francesco F., Raffaele C., Biagina C., Paolo P., Giorgio V. (2009). Composition and characteristics of ass's milk.
- Ezedeen, H. (1998). The importance of donkey in a restocking programme in Eritrea: Ministry of Agriculture, Gash-Barka Zone, Eritrea.
- Fantuz, F., Vincenzetti, S., Polidori, P., Vita, A., Polidori, F., & Salimei, E. (2001). Study on the protein fractions of donkey's milk: In: Proceedings of the XIV A.S.P.A. Congress (pp. 635–637). Firenze, Italy.
- FAO, (1990). The technology of traditional milk products in developing countries: FAO Animal Production and Health Paper 85. FAO, Rome, Italy. 333 pp.
- FAO, (2005). FAOSTAT Data: <http://faostat.fao.org/site/409/Dektop>.
- Felleke, G. and Geda, G. (2001). The Ethiopian Dairy development policy: A draft policy document. Ministry of Agriculture/ AFRDRD/AFRDT Food and Agricultural Organization of United Nations/SSFF. Addis Ababa, Ethiopia.
- Getachew Felleke (2003). Milk and dairy products, post-harvest losses and food safety in sub-Saharan Africa and the Near East: Assessments Report on the Dairy Sub-sector in Ethiopia. Action Programme for the Prevention of Food Losses, FAO, Rome.
- Giosuè, C., Alabiso, M., Russo, G., Alicata, M.L., Torrisi, C. (2008). Jennet milk production during the lactation in a Sicilian farming system.
- Giuffrida, M.G., Cantisani, A., Napoletano, L., Conti, A. & Godovac- Zimmerman, J. (1992). The amino-acid sequence of two isoforms of alactalbumin from donkey milk is identical: *Biological Chemistry Hoppe-Seyler*, 373, 931–935.
- Goldman, A.S., Anderson, D.W., Sellers, W.A., Saperstein, S., Kniker, W.T., Halpern, S.T. (1963). Oral challenge with milk and isolated milk proteins in allergic children: *Pediatrics* 32, 425–443.
- Guo, H.Y., Pang, K., Zhang, X.Y., Zhao, L., Chen, S.W., Dong, M.L., Ren, F.Z. (2007). Composition physicochemical properties, distribution, and amino acid profile of donkey milk: *Journal of dairy Science*, 90:1663-1643.
- Groziak, S.M, Miller, G.D. (2000). Natural bioactive substances in milk and colostrums: effect on the arterial blood pressure system. *Br J Nutr* 84(1):119–25.
- Herrouin, M., Molle, D., Fauquant, J., Ballestra, F., Maubois, J.L., Leonil, J., (2000). New Genetic Variants Identified in Donkey's Milk Whey Proteins. *J. Protein Chem.*; **19**: 105.

- Horrobin, D.F. (2000). Essential fatty acid metabolism and its modification in atopic eczema: *American Journal of Clinical Nutrition*, 71, 367S–372S.
- Iacono, G., Carroccio, A., Cavataio, F., Montalto, G., Soresi, M., and Balsamo, V. (1992). Use of donkey's milk in multiple food allergy: *J. Pediatr. Gastroenterol. Nutr.* 14:177–181.
- Ibrahim, H. R., and Aoki, T. (2003). New promises of lysozyme as immune modulator and antimicrobial agent for nutraceuticals: *Foods and Food Ingredients Journal of Japan*, 208, 361–374.
- Jiao, B. H., Chen, J. H., Miao, W. M., Wang, L. H., Zhu, Y. P., & Miao, H. N. (2004). Identification and biological characterization of angiogenic and tumour growth inhibitors derived from *Sinica cetorhinus maximum* cartilage: *Marine Drugs*, 2,30–38.
- Kathy, M. and Zahra, A. (1998). Gender issues in donkey use in rural Ethiopia:
- Ketema, H. (2000). Dairy development in Ethiopia: In the role of village dairy co-operatives in dairy development: SDDP (smallholder Dairy Development Project) Processing's, MOA (Ministry of Agriculture), Addis Ababa, Ethiopia.
- Luthfor Ahmed, SK. Nazrul Islam, Nazrul Islam Khan and Syeda Nilufer Nahid (2004). Vitamin C Content in Human Milk (Colostrum, Transitional and Mature) and Serum of a Sample of Bangladeshi Mothers: Institute of Nutrition and Food Science, University of Dhaka, Dhaka-1000, Bangladesh. *Mal J Nutr* 10(1): 1-4.
- Malacarne, M., Martuzzi, F., Summer, A. and Mariani, P. (2002). Protein and fat composition of mare's milk: some nutritional remarks with reference to human and cow's milk. *International Dairy Journal*, 12, 869–877.
- Mohammad I., Hamayun K., Syed Shah H., Rasool K. (2008). Physicochemical characteristics of various milk samples available in Pakistan: Institute of Chemical Sciences, University of Peshawar, Peshawar-25120, Pakistan.
- Meftuh, A.B., Tapsoba, L.P. and Lamounier, J.A. (1990). Breast feeding duration on Ethiopian women in Southern California: *Journal of Tropical Pediatrics*, 36(4):203-4.
- Miranda, G., Mahe, M. F., Leroux, C., & Martin, P. (2004). Proteomic tools to characterize the protein fractions of Equidae milk: *Proteomics*, 4, 2496–2509.
- Morgante, M., Ranucci, S., Pauselli, M., Casoli, C., Duranti, E., (1996). Total and differential cell count in milk of primiparous Comisana ewes without clinical signs of mastitis: *Small Rum. Res.* 21:265-271

- Monti, G., Bertino, E., Muratore, M.C., Coscia, A., Cresi, F., Silvestro, L. (2007). Efficacy of donkey's milk in treating highly problematic cow's milk allergic children: an in vivo and in vitro study. *Pediatr. Allergy Immunol.* 18:258-264.
- O'Connor, C.B. (1992). Rural smallholder milk production and utilization and the future for dairy development in Ethiopia.
- O'Connor, C. B. (1995). *Rural Dairy Technology: ILRI Training Manual 1*. International Livestock Research Institute, Addis Ababa, Ethiopia.
- Oftedal, O.T. and Jenness, R. (1988). Interspecies variation in milk composition among horses, zebras and asses (*Perissodactyla: Equidae*): *J. Dairy Res.* 55:57–66.
- Pearson, R.A., Muir, C., and Farrow, M. (2006). *The Future of Working Equines: The Donkey Sanctuary*. Addis Ababa University, Ethiopia.
- Peeler, E.J., Green, M.J., Fitzpatrick, J.L., Morgan, K.L., Green, E. (2000). Risk factors associated with clinical mastitis in low somatic cell count British dairy herds: *J. Dairy Sci.* 83:2464-2472.
- Pieter Walstra, Jan T. M. Wouters, Tom J. Geurts (2006). *Dairy Science and Technology: Second Edition*.
- Salimei, E., Fantuz, F., Coppola, R., Chiofalo, B., Polidori, P. and Varisco, G. (2004). Composition and characteristics of ass's milk: *Anim. Res.* 53:67–78.
- Samia M.A., Abd Elrahman, Said Ahmad, Ibtisam E.M., El Zubeir., O.A.O. EL Owni and M.K.A. Ahmed (2009). Microbiological and Physicochemical Properties of Raw Milk Used for Processing Pasteurized Milk in Blue Nile Dairy Company (Sudan). *Australian Journal of Basic and Applied Sciences*, 3(4): 3433-3437, ISSN 1991-8178.
- Saxena, M.M., Katpatal, B.G. and Pandey, H.S. (1997). Study of milk constituent and their yield in Holstein-Friesian cows: *Indian Journal of Animal Production and Management* 13 (3):127-130.
- Schaafsma, G. (2003). Nutritional significance of lactose and lactose derivatives: In H. Roginski, J. W. Fuquay, and P. F. Fox (Eds.). *Encyclopedia of Dairy Science* (Vol. 3, pp. 1529–1533). London: Academic Press.
- Stephen, D. and Simon, P. (2006). Donkey milk holds the secret to skin health.
- Taha, N.M. and Kielwein, G. (1990). Pattern of peptide-bound and free amino acids in camel, buffalo and ass milk: *Milchwissenschaft* 45:22–25.

- Taylor, S.L. (1986). Immunologic and allergic properties of cow's milk proteins in humans: *J. Food Prot.* 49 (3), 239–250.
- Tesse, R., Claudia, P., Serena, B. and Lucio, A. (2009). Adequacy and tolerance to ass's milk in an Italian cohort of children with cow's milk allergy: Department of Biomedicine of the Developmental Age, Pediatric Unit "S. Maggiore", University of Bari, Bari, Italy.
- Van-den, B.J.C. (1988). Dairy technology in the tropics and subtropics: Pudoc, Wageningen, The Netherlands. 290 pp.
- Vincenzetti, S., Polidori, P., Fantuz, F., Mariani, P.L., Cammertoni, N., Vita, A., Polidori, F. (2005). Donkey's milk caseins characterization: *Ital. J. Anim. Sci.* 4(Suppl. 2):427-429.
- Vincenzetti, S., Polidori, P., Mariani, P., Cammertoni, N., Fantuz, F., Vita, A. (2007). Donkey's milk protein fractions characterization: *Food Chemistry* 106 (2008) 640–649.
- Webb B H, Johnson A H and Alford J A. (1974). *Fundamentals of dairy chemistry*: 2nd edition, AVI Publishing Company Inc, Westport, Connecticut, USA. 929 pp.
- White, C.H. (2001). Testing milk and milk products: In E. H. Marth and J. L. Steele (Eds.), *Applied Dairy Microbiology* (2nd ed., pp. 645–680). New York: Marcel Dekker.
- Yang, H. F., L. Ma, X. G. Zhao, and D. Q. Su. (2006). Studies on the development and exploitation of donkey milk: *Chin. Food Nutr.* 4:22–24.
- Young, W.P and George, F.W.H. (2006). *Handbook of Milk of Non-Bovine Mammals*: Blackwell publishing.
- Zegeye, Y. (2003). imperative and challenges of dairy production, processing and marketing in Ethiopia: In: Joger Y and Gebry G (eds), challenges and opportunity of livestock marketing in Ethiopia. Proceeding of the 10th Annual conference of the Ethiopian society of Animal Production (ESAP) held in Addis Ababa, Ehiopia, 22-24 August 2002. ESAP, Addis Ababa, Ethiopia. pp.61-67
- Zhang, X.Y., Zhao, L., Jiang, L., Dong, M.L. and Ren F.Z. (2008). The antimicrobial activity of donkey milk and its micro flora changes during storage: Beijing Functional Dairy Laboratory, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China.