STUDIES ON THE QUALITY OF MARKET MILK AND DEVELOPMENT OF LOW COST MILK PRESERVATION TECHNOLOGY FOR RURAL FARMERS

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Registration No. 6249
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Abdul Hamid

Department of Dairy Science
Bangladesh Agricultural University, Mymensingh

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Ph.D. DISSERTATION

Approved as of style and contents

Professor Dr. Md. Nurul Islam
Chairman
Supervisory Committee

Professor A. K. M. Abdul. Mannan
Member
Supervisory Committee

Professor Dr. Md. Afzal Hossain
Member
Supervisory Committee

Chairman of the
Examination Committee
Bangladesh Agricultural University
Mymensingh

November, 2005

DECLARATION

I hereby declare that, the experiments included in the dissertation have been solely executed by me and the dissertation or any part of it has not been submitted for any other degree to any University, Institute or elsewhere. All sources of information are shown in the text and listed in references. The assistance and help received during the courses of investigation have duly been acknowledged.

The Author

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LIST OF CONTENTS

LIST OF CONTENTS (CONTD.)

LIST OF CONTENTS (CONTD.)

Page No.

LIST OF CONTENTS (CONTD.)

LIST OF CONTENTS (CONTD.)

Page No.

LIST OF TABLES

LIST OF TABLE (CONTD.)

LIST OF TABLE (CONTD.)

LIST OF FIGURES

LIST OF FIGURES (CONTD.)

LIST OF FIGURES (CONTD.)

LIST OF APPENDICES

LIST OF APPENDICES TABLE

LIST OF ABBREVIATION AND ACRONYMS USED

ANOVA	=	Analysis of Variance
APHA	=	American Public Health Association
Apporx.	=	Approximately
BAU	=	Bangladesh Agricultural University
BDP	=	Bangladesh Dairy Plant
c.f.u.	=	Colony Forming Unit
COB	=	Clot-on-boiling
Conc.	=	Concentration
D	=	Day
Deg.	=	Degree
Dr.	=	Doctor
Eg.	=	For example
et al.	=	Associates
F	=	Fahrenheit
F	=	Fat percentage of milk by Babcock method
Fig.	=	Figure
G	=	Gran
GDP	=	Gross Domestic product
H	=	Hour
KQ	=	Keeping quality Laboratory
Lab.	=	Laboratory
LP.	=	Lactoperoxidase
GOB	=	Government of Bangladesh
CLR	=	Corrected lactometer reading
LGE	=	Lactoperoxidase/garlic extract/ethanol
ml.	=	Mililiter

LIST OF ABBREVIATION AND ACRONYMS USED

N	=	Normality
NaOH	=	Sodium Hydroxide
No.	=	Number
NS	=	Non significant
PPC	=	Post Processing Contamination
P<0.001	=	Significant at 0.1% level
P<o.ol	=	Significant at 1% level
P<0.05	=	Significant at 5% level
Ppm	=	Parts per million
Sd	=	Standard deviation
SNF	=	Solids-not-fat
Sp.gr.	=	Specific gravity
SPC	=	Standard plate count
Temp.	=	Temperature
TS	=	Total Solids
TVC	=	Total viable count
USA	=	United States of America
VRBA	=	Violet Red Bile Agar
%	=	Per cent
<	=	Is less than
>	=	Is greater than
±	=	Plus or Minus
°C	=	Degree Celsius

ABSTRACT

The research project was carried out at the Dairy Technology and Microbiology Laboratory of the Department of Dairy Science, Bangladesh Agricultural University, Mymensingh to evaluate the quality of market milk collected from some selected areas of Bangladesh and at the same time to monitor the feasibility of using some traditional rural technology used by farmers' to improve the storage life of milk. Attempts were also made to find out low cost milk preservation technique which could be used by village farmers' without having any risk of health hazards. To achieve the objectives of the project a series of experiments were conducted out of them two were on milk quality and others four were on milk preservation by various means. First experiment was carried out in some selected areas of Mymensingh district and the second experiment was carried out in Northern part of the country (Sirajgonj and Pabna district). In both experiments, milk samples were collected from different sources like city centre, local makets, farmers' house and organized dairy farm etc. to evaluate the quality of milk. Some physical, chemical and microbiological tests were conducted to evaluate the quality of milk. From the result of both experiments it was found that wide variations were found within different samples regarding quality. On the basis of physical and chemical parameters it was found that milk produced at Bangladesh Agricultural University Dairy Farm was superior to other milk samples. But microbiological parameters indicated that total viable bacterial cound and coliform bacterial count for all samples were higher than normal value indicating that proper hygienic measures were not taken during milking, handling and transportation of milk. Third experiment was conducted to see the feasibility of using sodium bicarbonate on the keeping quality of milk. In this experiment fresh milk samples was preserved by adding 0.10, 0.15 and 0.20 per cent sodium bicarbonate on the basis of weight of milk samples. Shelf-life of the samples were judged with the help of acidity and COB test. It was found that at room temperature 30–32^{0}C control milk sample spoiled at 12th hour but 0.10, 0.15 and 0.20 per cent sodium bicarbonate added samples spoiled at 13, 14 and 16th hour respectively indicating that this chemical could be used for short time preservation of milk. The fourth experiment were carried out to monitor the effects of containers on shelf-life of milk. Fresh whole milk samples were kept at room temperature in six different types of containers namely stainless steel, aluminium, glass, plastic, tin and earthen containers. It was found that stainless steel and aluminium containers were good for storage of milk followed by glass and plastic containers. In tin and earthen containers milk sample spoiled rapidly. In fifth experiment attempts were made to evaluate the effectiveness of adding banana leaf on shelf-life of milk. But it was found that banana leaf could help to prevent agitation but could not improve the storage life of milk. Sixth experiment was carried out to see whether the shelf-life of whole milk samples could be increased for a while by adding fresh water. For this purpose 10, 20 and 30 per cent of fresh cold water was added with milk samples and found that by adding 20 to 30 per cent water with milk shelf-life could be extended 3 to 4 hours more than the normal shelf-life of milk.

CHAPTER 1
INTRODUCTION

Chapter 1

INTRODUCTION

Bangladesh has a unique geographical position with an area of 1,48,793 sq. km. and geographical location between 20°34' N to 26°38' North latitude and 88°01' to 92°41' East longitude. It is located in Southern Asia, bordering the Bay of Bengal, between Myanmar and India. Total area is smaller than Wisconsin, USA. Bangladesh is the largest deltaic plain in the world (Anon. 1981). Country's average summer temperature varies from 21-34°C and winter temperature ranges from 11-29°C. The average monsoon rainfall is from 1196 mm to 3454 mm with the highest humidity in July (99%) and lowest in December (36%) (BLRI, 2001).

Bangladesh in one of the most densely populated countries in the world having 13.40 million people. The density of human population per square kilometer is about 755. Out of the total population about 80 percent lives in the rural area of which 70 percent are directly or indirectly involved in agricultural operations and around 47 percent of the total human population live below the poverty line. Predominantly agriculture is the back-bone of the national economy of the country. The contribution of Agricultural sector to the national GDP is 61.31 percent of which 12.47 percent comes out from livestock alone. Livestock rearing is an integral part of the farming system in Bangladesh.

Livestock and its product provide direct cash income, since they are living bank for rural farmers and are critical to agricultural intensification where it provides power and manure as fertilizer and fuel. Dairying is nearly always a part of a mixed farming system. This has a direct impact on employment opportunity, fosters welfare to the rural farming community, income generation, poverty alleviation and availability of animal protein (Sadullah, 2001). By quantifying livestock including

poultry shows that dairying is the predominant source of income generation (Miyan, 1996). Although the supply of domestically produced animal protein has increased by about 1.2 percent annually (D.L.S, 2000). The people suffer from acute shortage of livestock products, milk (85%), meat (89%) and egg (75%). Presently there are about 23.4 million of cattle, 0.82 million of buffaloes, 33.5 million goats, 1.11 million sheeps, 138.2 million chicken and 13.0 million ducks (D.L.S, 2000).

Annually, they provide 95 percent of draft power, 0.62 million metric tons of meat, 1.6 million metric tons of milk, eggs 134 thousand metric tonnes or 572 crore piece, 80 million metric tons of organic manure and 20 percent of income generation (D.L.S-2000). In our country, contribution of the total agricultural sector to the GDP was 31.5 percent in 1999 and the contribution of livestock to the Agriculture GDP was 3.3 percent (BBS, 2000). To meet the increasing demand of milk, meat and eggs, average annual productivity of the native animals and birds are required to be increased from the present level of 221 kg to 500 kg milk/cow, 50 kg to 100 kg meat/animal and 45 to 100 eggs/bird (BLRI, 2001).

In comparison with other country like Sri Lanka, contribution of the total agricultural sector to the GDP was 20.7 per cent in 1999 and the contribution of livestock sector to the agriculture GDP was 8 per cent (Bandara, 2001). In Thailand, milk production comprises only about 20 per cent of the total consumption, the rest had to be imported, (Chantalakhane and Skunmun, 2001). In Pakistan, milk and milk products are the most important food items with an annual combined consumption of more than 12 million tons. Pakistan maintains the highest consumption level per capita of all Asian countries (Raja, 2001). In India, over 70 per cent of all rural holds depends on livestock farming for supplementary incomes (Kurup, 2001). India had some 204 million cattle and 84 million buffaloes in 1992. Cattle production grew by 33 per cent during the four decade (between 1985-1992), however, the rate of growth slowing down visibly over the past decade (0.48 per

cent per annum between 1987-1992), while buffalo population almost double over same period, growing much faster (almost 2 per cent per annum in during 1987-1992) (Kurup, 2001).

In Bangladesh the production of milk is very scantly. In our country most of the cows are indigenous (non-descript) type. The production of milk from these cows is about one liter per day. Cattle and buffalo supplies about 99 per cent of total milk production in our country (BBS, 1997). Only 1.57 million metric tons of milk per year is produced in Bangladesh and the per capita per day availability of milk is 37g as against the demand of 240g (Sadullah, 2001). It was also reported by Sadullah, 2001 that the average milk yield per cow is 1.5 litre for local cows and 2.5 litre from crossed cows. According to a report of Government of Bangladesh (GOB, 1999), average per capita availability of milk is 35 ml per day whereas per capita requirement of milk is 250 ml. Annual total requirement of milk is 11.65 million metric tons whereas total annual production is 1.62 million metric tons and total annual shortage of milk is 10.03 million metric tons (87%). Although there has been an improvement of the livestock sub-sector due to improvement in private sector but still exits a huge deficit. Because of the continuous increase of population, the deficit appears to be persisting in the foreseeable future. The deficit is met up by importing 20-30 lakh or more metric tons of powdered milk every year. This amount of milk contributes only the consumers require 34 per cent of the total milk. So it is necessary to increase the milk production to mitigate the requirement. Most of the people of our country have been suffering from protein calorie malnutrition. To make people more productive, the public health must be improved. To maintain a healthy population, the animal protein supply should be increased.

Milk is a worldwide popular nutritious food. Everybody likes this food. It contains most of the nutrients required for normal functioning of the body system. It is established that the milk and milk products are the most important healthy food for

human being. For the newly born infant or animal, milk is an almost complete and well-balanced food.

The main constituents of milk are- (1) water (ii) protein (iii) fat, (iv) carbohydrate (Milk sugar or lactose) and (v) ash. The constituents may vary with the breed, type of feed, stage of lactation, season, age of the cow etc. and also between individuals of the same breed.

Besides, the above constituents' milk also contains considerable amount of fat-soluble vitamins (A, D, E & K) and water-soluble vitamins (B complex and C).

Milk fat often called "Butter fat" is commercially, the most valuable constituent of milk. Milk fat has a special significance in nutrition due to presence of wide range of fatty acids. Fat in milk serves as the concentrated source of energy and each gram of fat supplies 9 calories, an energy value of 2.25 times as high as that of either protein or the carbohydrates. It Is a carrier of fat-soluble vitamins and helps in lactose assimilation.

The protein of milk in not a single compound but includes two major proteins and small quantities of others. Of these, casein constitutes about 80 percent of the total protein and lactalbumin 18 percent. A third protein recognized as present in milk in lactoglobulin. The essential amino acids like tryptophan and lysine are present in large quantity in milk, which are deficient in vegetable protein. Besides these glutamic acid is present in cows milk three times higher than human milk, which result a reduction of cholesterol in blood. Oratic acid of milk protein improves liver detoxification. Another content taurine is responsible for the development of immature brain.

Milk sugar, commonly designated by the chemist as "lactose" is found only in milk. The combination of 1 molecule of galactose and 1 molecule of glucose form it Galactose is essential for synthesis of galactosides, a constituent of central nervous system and therefore lactose may be considered as a brain food and are indispensable carbohydrate for growth and development of central nervous system of mammalian young.

Milk is a good source of calcium and phosphorus, which are important in the formation of bone and teeth. Calcium aiding in the contraction and relaxation of heart muscles including clotting of the blood to prevent fetal bleeding and maintain buffer capacity of blood.

All vitamins essential for human health and nutrition are available in milk some are present in large quantity then human requirement. There is general agreement that as consumers, we want clean, wholesome and nutritious food that is produced and processed in a sound, sanitary manner and is free from microbial pathogens. For this reason, the nutritive value of milk depends on its freshness, cleanliness, purity and wholesomeness. The milk and milk products having these characteristics are consumers demand and for this reason at the time interval between milk collection from the small farmers to the consumers is primarily most important to ensure fresh, clean pure and wholesome milk and milk products to the consumers. In Bangladesh condition usually milk is being supplied to the consumers from the urban and rural areas by Goalas. They collect also milk from different small farmers as well as from the local markets. In Bangladesh we donot have enough facilities for producing high quality milk as because lot of factors are associated with this. Farmers have very little knowledge about the scientific methods of milking and milk preservation. At the same time transportation and marketing facilities are also poor which leads to the delivery of poor quality milk to the consumers. Due to these circumstences we donot have enough idea about the quality of milk we are consuming everday. In

order to produce high quality milk it is always necessary to check the quality of milk available in local markets.

Determination of the levels of micro-organisms chemical composition and adulterants in milk is essential to know its quality for a successful dairy operation and to save the public health. A dairy man must not only have relatively high production of milk per cow but he must also produce quality milk for public health to ensure an immediate market for his milk and long term demand for milk by the consumers.

In rural condition flavour and colour is the primary indicator of the quality of milk. But the established dairy industries use bacterial count; sediment test and other chemical tests are as measures of the quality of milk. Here quality milk means, the milk which is

i) Free from pathogenic bacteria and harmful toxic substances.

ii) Free from sediment and extraneous substances.

iii) Of good flavour

iv) With normal composition.

v) Adequate in keeping quality,

vi) Low in bacteria count (Foster et al., 1958).

Quality milk production requires clean, healthy and well-fed cows, clean, dry well ventilated barns and clean sanitized utensils. The flavours impair the quality of milk from taste stand point and these are oxidized flavour, rancid flavour, feed and weed flavour, cowy flavour, malty flavour and salty flavour. The rancid flavour is due to the break down of milk fat by the enzyme lipase. Feed flavour is caused by feeding onion, garlic, silage, fishmeal etc. just before milking. The malty or acid flavour in

milk is caused by unclean milk handling equipments or by poor cooling of milk that occurs when a large number of bacteria are present in milk. The bacterial count indicates how milk has been handled. The flavour of milk also largely depends on tile type of feed given before milking and hygienic condition of the barnyard. The dirt found in milk is also an important factor for quality control problem and also for selling of milk. Acidity of milk indicates whether it is sour or soon likely to become sour. Normal composition of milk preferably, percentage of fat is also an important factor for chemical quality of milk.

In our country, milk is marketed on the basis of volume and not on the basis of milk fat, milk protein, or milk solids-not-fat content. This provides a wide scope of adulteration of the milk by various means which causes adverse affect on the consumers and dairy products and by-products.

The nutritive value of milk also depends on its freshness cleanliness, purity and wholesomeness. The milk and milk products having these characteristics are consumer's demands and for this reason milk examination is most important to ensure fresh, clean, pure and wholesome milk and milk products to the consumers.

Reports are available on the various works done on the quality of market milk in different countries of the world under the condition existing in their own localities. Yadav and Saraswat (1982) reported the composition of milk of the market of Varanasi town India. They found market samples had abnormal colours and taste but normal flavour. The market samples had lower specific gravity and higher acidity. Fat, total solids, solids-not-fat contents of market milk were lower than the control sample. Orlajenson and Plattner (1969), also reported that quality market milk of their own localities were poor. A few work has been done on the quality market milk in Bangladesh. Islam *et al* (1984) studied the quality of milk available in local markets of Mymensingh town and Bangladesh Agricultural University

Dairy Farm. They showed that the quality of milk supplied by milk vondors to local market was poor.

Lack of preservation facilities are also serious constrains in the field of dairy production. Although milk is a highly nutritious food for human being but it is a good food for microbes also. It can undergo spoilage due to action of various microorganisms. Lactose is broken down to glucose and galactose by microbial enzyme lactase and finally lactic sold is produced from glucose.

$$C_{12}H_{12}O_{11} + H_2O \text{ (+bacteria)} \xrightarrow{\text{Lactase}} 4C_3H_6O_3 \text{ (Lactic acid)}$$

If the concentration of lactic acid (acidity) increases, then the quality of milk is deteriorated. Established dairy farms have modem facilities for milk preservation but the small-scale farm have no such type of facilities for milk preservation. As a result, a considerable amount of milk undergoes spoilage due to lack of preservation facilities. In order to increase the storage life of milk, heat treatment, cold treatment, chemical methods and some other special methods are applied by dairy farmers.

In Bangladesh, the production of milk takes place in a very disorganized way, Although there are few milk pockets where surplus milk is readily available, but this perishable product has neither received particular attention for by hygienic distribution to the consumers nor have been preserved scientifically. Lack of proper transportation and inadequate refrigeration facilities as well as the problems associated with the nature of the product, may create difficulties for its uniform distribution to urban areas from the remote village pockets.

Historically milk first received heat treatment to increase the shelf life. Heat treatment of milk completely destroys the organism of milk borne disease. When it became evident that milk could serve as source of food borne illness, heat treatment became a necessary safeguard. There were epidemics of typhoid fever, scarlet fever,

diphtheria and infantile diarrehoea, as well as out breaks of tuberculosis and other illness resulting from drinking of raw milk Today rnuch with view credit goes to heat treatment by pasteurization to destroy all pathogenic organisms, to reduce the total microbial loads extending the storage life and inactivating enzymes that can affect milk flavour adversely. Although milk is a delicately flavoured and easily changed food, but the mild heat treatment by pasteurization technique not only kills pathogens but also Improve the keeping quality of milk without affecting deleteriously the appearance, flavour, nutritional properties or creaming of milk.

Ultra-heated treated (UHT) milk was developed to meet the demand for milk which was stable for extended periods at room temperature and yet was free of the unpleased taste associated in continental Europe (Verman and Sutherland, 1996). At present, Milk Vita and Aarong are using UHT method and supplying such type of milk in local markets.

Most milk plants prefer to sell heat-treated milk in closed sealed container, thus preventing the product from being recontaminated or adulterated. However, the consequences of contamination of unpacked milk should be overestimated, at least not in those regions where in customary to boil the milk before use. The costs of packaging pasteurized and sterilized milk are high and as such they increase the cost of production considerably. Therefore, serious consideration must be given to whether the advantages of packaged milk justify the higher price, especially because the lower income groups may be unable to pay such a price to buy sufficient amounts of milk. Moreover, for practical reasons, a milk plant must limit the number of packages of different size and since the costs of packaging increase with decreasing capacity of the containers, very small packs will not be very popular in the plant. Contrary to the views of the plant the consumer, especially if poor, may wish to by very small amounts. (Van den Berg, 1990).

From the above discussion it is clear that we donot have enough idea about the quality of our market milk. For this reason it is urgently needed to analyse the whole milk by collecting from different areas of the country. Similarly farmers have limited scope for milk preservation. Most of the farmers of our country is poor, they donot have the refrigerated facility, moreover, electricity is not available in all rural areas. Farmers are using some indigenous techniques for this purpose. So, it is very much important to study the scientific basis of their techniques and at the same time an attempt should be made to develop suitable milk preservation technology appropriate for rural areas. Hence, the present study was under taken with the aim of investigating the physical, chemical and bacteriological qualities of market milk produced in the rural areas of Bangladesh as well as to monitor the shelf-life of milk at room temperature under our climatic condition. At the same time attempts would also be made to develop suitable technology for milk preservation under rural condition.

Taking into account of the facts, the present experiment was undertaken to achieve the following objectives:

a) To study the quality of market milk available in different areas of Bangladesh

b) To detect specific adulterants (if any) in market milk

c) To evaluate the feasibility of using indigenous techniques to enhance the keeping quality of milk

d) To develop a low cost technology of milk preservation for rural farmers of Bangladesh

e) To recommend farmers about the appropriate technology to be used in our country condition to increase the keeping quality of milk.

CHAPTER 2

REVIEW OF LITERATURE

Chapter 2

REVIEW OF LITERATURE

The purpose of this section is to review the findings of past research having relevance to the present study. Various kinds of research works have been done in the past by many scientists of the world regarding quality of market milk and their shelf life. Very limited research works were conducted in Bangladesh conditions. Some of the important research findings of them related to milk quality and preservation techniques of milk are reviewed in this section. This section has been divided into several sub sections for easy understanding.

2.1. Physical parameters of milk

Eackels *et al.*1951) mentioned that milk ranges in cotour from a bluish white to a golden yellow, depending upon the breed of animal, the kind of feed and the amount of fat and sotids present. In large quantities milk appears entirely opaque while in thin layers it is slightly transparent. Milk from which the fat has been removed or, milk which is low in fat percentage shows a bluish tint. The white cotour of milk is due to the reftection of light by the disrpersed fat globules, the calcium caseinate and the colloidal phosphate. They also reported that specitic grarity of normal milk lies within the ranges of 1.027 to 10035 with an averase value of 1.032.

Ward *et al.* (1956) reported that the age, stage of lactation, milk yield, time of lucerne silage feeding and heavy feeding molasses, milo did not appear to affect milk flavour. Garlic or onion placed in the rumen caused a stronger off flavour.

Olson (1956) reported that the physical disturbed condition of the cow may cause an. objectionable flavour of milk. Feed flavours which are carried in the blood of the cow frequently cause objectionable flavour and odour in the, milk. The weeds or feeds are wild onions, French weeds, bitter grass, bitter weeds, green eye, rape etc.

Judkins and Keener (1960) studied on the normal taste, colour and flavour of milk produced under sanitary condition. They observed that the normal taste of milk was slighty sweet, flavour was mild aromatic and normal colour of milk was yellowish white. The sweet taste comes from the lactose lactose and butter fat. The colour of milk comes generally from the carotene content of milk.

Lampert (1970) stated that Guernsey and Jersey breed can transfer more carotene, the pigment that actually impart yellowish colour to milk from their feed to milk fat as compared to Holstein, Ayrshire and other breeds and hence the colour of milk from Guernsey and Jersey is deeper i.e. more yellowish. He also reported that the colour of milk varies upon fat, solids-not-fat (S.N.F) and the size of the fat globule.

Bandler (1971) stated that both flavour and odour are affected by feeding of cow some spicy feeds like onion, garlic, etc. which add off flavour and odour in milk.

Folley *et al.* (1972) reported that "salty" milk is frequently produced in the last stage of lactation by cows. A "cowy" flavour is found in milk from cows with "ketosis". A burny taste occurs in milch cows, which were housed in poorly ventilated shed. The milk may have a strong dis-agrreable taste caused by garlic, onion or strong silage.

Foley *et al.* (1972) found the cause of abnormal flavour and taste of milk. They reported that salty milk is frequently produced by cows in the final weeks of lactation and by cows with chronic mastitis. A cowy flovour is found in milk from cows with ketosis. The barny taste in the milk is caused by cows housed in poorly ventilated shed.

Baevre *et al.* (1976) carried out an experiment to see the effect of addition of various types of fat on flavour of milk. They reported that, flavour of milk is affected by acidity, feeding various types of fat like sunflower, soya been, rapeseed oil etc. had produced rancid flavour in milk.

Dehury *et al.* (1977) evaluated the market milk at Bhehanaswar of India by collecting milk samples from established dairy farm, local milkman, local shops,

milk collection centres and pasteurized milk booths. They reported that all milk samples from established dairy farms and pasteurized milk booths had normal colour, consistency, flavour, odour and taste. Out of 100 samples from local gowala, 33 were bluish, 60 yellowish, 7 soapy and 15 had a whitish taste. Out of 85 samples from local shops, 48 had a thin consistency with a blusish or whitish colour except for 5 which were brown; 5 were rancid and 2 had a charred smell and 2 were soapy and out of 123 samples from milk collection centres 110 were normal, 9 were charred smell and 4 with cowy odour.

Yadav *et al.* (1982) examined the market milk of Varanasi Town, India. They reported that market milk samples had abnormal colour and taste but normal flavour.

Islam *et al.* (1984) studied the physical and chemical qualities of milk ofBangladesh Agricultural University (BAU) dairy farm and market milk of Mymensingh town. They stated that out of 35 samples from BAU dairy farm, all samples wereyellowish white in colour, slightly sweet in taste and normal in flavour. The average specific gravity, fat and acidity were 1.031, 4.80% and 0.15% respectively. But from 35 samples the market milk of Mymensingh town, 25 were white,7yellowishwhiteand 3bluishincolour.The average specific gravity, fat and acidity of market milk of Mymensingh town were 1.026, 3.02% and0.14% respectively.

Ghafoor *et al.* (1985) studied physical-chemical post-milking changes at room temperature (30-35^{0}C) in July with 50 samples of cow's milk and 50 of buffaloes' milk. Milk from both species showed no changes in appearance, taste, or odour during the first 4 h. Initially cow's milk had a titratable acidity of .16% and a p^H of 6.8, changing to 0.75% and p^H 5.3 after 12 h. at room temperature. Corresponding figures for buffaloes milk were 0.15% titratable acidity and p^H 6.9 initially, 0.76% and p^H 5.4 after 12h It was observed that raw milk can be stored at room temperature for 4 h. in July and Aug. without deterioration in quality.

Duncan *et al.* (1991) measured that rancidity scores and ADV increased with storage time. Major free fatty acid concentrations increased as ADV increased ($r= 0.93$, $p=0.0001$) for farm milk samples but correlations was low ($r= 0.27$, $p= 0.40$) for laboratory-prepared rancid samples.

Reinheimer *et al.* (1993) observed that the major flavour defects were associated with microbial processes. At 12°C, there was a higher incidence of thermoduric microflora at spoilage. The flavour was affected mainly by acid development and proteolytic activity.

Senapoty *et al.* (1995) carried out a comparative study on raw buffalo milk samples produced in `organized (composite livestock farm) and non organized sectors (rural areas of co-operative milk producers union) at the milk distribution point around Jabalpur, India and reported that organoleptic evaluation of milk samples showed a significant ($P<0.05$) variation in milk quality.

Mizanur (1995) found that the specific gravity of Manikganj chilling centre, Tangail chilling centre, Takerhat pasteurized plant and Baghabarighat, Dairy Plant were 1.0248, 1.0229, 1.0246 and 1.0273.

Gob *et al.* (1995) stated that the agitation increased FFA levels of milk, with agitation by nitrogen gas having a greater effect than stirring, and agitation twice having effect than a single agitation.

Aakuzwa *et al.* (1995) determined milk aroma from Holstein cows heated to 63°C for 30 min, 75°C for 15 s (pasteurized milk) or 120°C for 3s (UHT milk) and the aroma was compared with that of unheated control milk. The strength of the milk-like aroma decreased as the heating temperature increased; however, milk heated to 63°C for 30 min had a similar strength aroma to that of control milk. Pre incubation of milk at 63°C for 30 min before heating to higher temperatures made the milk-like aroma resistant to heat treatment. The strength of the aroma as evaluated when opening milk containers decreased as the heating temperature increased. When

control and heated milk were fractionated, the milk-like aroma appeared to be located in the milk fat globule membrane.

Manzoor Quadir (1996) carried out an experiment on the assessment of chemical qualities of milk produced by primary cooperative societies (Milk Vita). He found that the specific gravity of Rawtara, Pesombari and Briangaru societies were 1.027, 1.027 and 1.026 of Baghabarighat milk shed area.

2.2 Chemical parameters

Overman *et al.* (1929) conducted an experiment to determine the composition of Holstein, Ayrshire, Guernsey and Jersey cow's milk. They found that the average fat, solids-not fat and total solids content of milk were 3.55%, 8.96% and 12.41% for Holstein cows, 4.14%, 8.97% and 13.11% for Ayrshire cows 5.19%, 9.68% and 14.86% for Guernsey cows and 5.18%, 9.51% and 14.69% for Jersey cows. Turner (1936) found similar result with the similar breeds.

Kothawalla and Kartha (1939) analysed the average percentage of fat, solids-not-fat (SNF) and total solids (TS) of milk of Tharparkar and Hariana and found that the average fat, SNF and TS were 4.60%, 8.52% and 13.12% of Tharparkar breed and 4.60%, 8.66% and 13.26% of Hariana breed respectively.

Warner (1953) analysed Red Shindi cows and Zebu cattle milk and found the average fat, SNF and total solids of Red Shindi cows milk were 4.60%, 8.52%, 13.42% and the Zebu cattle milk 5.600/0, 8.70% and 14.30% of fat., SNF and total solids respectively.

Overman *et al.* (1953) carried out an experiment and showed that the chemical composition of Brownswiss cows milk and they found that the average fat, S.N.F. protein and ash were 3.97%, 9.16%, 3.52%, and 0.74% respectively.

Filiptovic (1953) also found that the average specific gravity, fat, protein and ash were 1.032, 3.91%, 3.33% and 0.72% respectively from Yugoslavia market milk.

Ghani *et al.* (1954) examined the average composition of our indigenous cows milk of different districts of East Bengal (Now Bangladesh). They reported that the average milk fat percentage of different districts varied from 4.4 to 6.8 and the SNF percentage from 8.40.to 9.99 and the verall average composition of East Bengal cow's milk was 5.4% of fat, 9.10% of SNF and 14.54% of TS.

Ghani and Rahman (1954) conducted an experiment to study the composition of milk of different districts of East Bengal (now Bangladesh). They observed that the average milk fat percentage of different districts varied from 4.4 to 6.8 and SNF percentage from 8.40 to 9.99 and overall average composition of native cows milk was 5.4% butter fat and 9.10% SNF and 14.54% TS.

Morihide (1956) stated that the average fat and solids-not- fat content of market milk was 3.07-3.47 and 7.71-8.16% respectively.

Armstrong (1959) studied the composition of milk of different dairy breeds. He found that average percentage of fat, solids-not-fat and total solids in milk were 3.49, 8.61 and 12.10 for Holstein, 4.02, 9.39 and 13.41 for Brown Swiss, 4.15, 8.96 and 13.11 for Ayrshire, 4.99, 9.32 and 14.31 for Guernsey and 5.51, 9.49 and 15.00 for Jersey cows.

Wahid (1960) studied the composition of milk of Sahiwal cows and found that it contained 4.0 to 6.0 percent butter fat.

Agarwala and Sharma (1961) found that addition of water, skimming of milk and both skimming and watering redacted the fat. He reported that addition of water not only involves the dilution of the milk but also the danger of introducing germs with polluted water. Mishra and Nayak (1962) in an experiment analysed the milk samples from Orissa and reported that fat, solids not fat and total solids of milk were 4.65%, 9.39% and 14.04% respectively.

Ito (1966) observed the variation in the quality of raw cows milk. He analysed 57000 samples of milk from April 1964 to March 1965 and the means with standard deviations obtained for specific gravity was 1.0304± 0.006, acidity 0.148 ±

.008%, fat 3.372 ± 0.118%, protein 2.971 ± 0.104%, lactose 4.388 0.062%, ash 0.687 0.026% and SNF 7.929 + 0.137 %.

Delforno *et al.* (1966) investigated 720 mixed samples from herds for Friesian cattle in Italy. They found that the specific gravity, T.S., fat, SNF and protein were 1.0311 (1.0297 to 1.0325), 12.30% (11.97% to 12.680/o), 3.65% (3.43% to 3.83%), 8.65% (8.47% to 8.87%) and 3.10% (2.96% to 3.26%) respectively.

Hossain (1968) determined the average percentage of fat, SNF, and TS of indigenous cows milk. He stated that the average percentage of fat, SNF and TS content of indigenous cows milk and their standard deviation were 4.60± 0.640% and 13.51± 0.896% respectively.

Yoshida (1969) tested 26 samples of market milk in Fukuyama. He reported that 14 out of 26 samples did not meet minimum legal standards (3.0% fat and 8.01 % S.N.F) in Japan. He found that the percentage of fat and S.N.F were 2.83-3.63% and 7, 72-8.44% respectively.

Lavania (1969) carried out an experiment to evaluate the physical and chemical quality of market milk at Baraut town of India by collecting 234 samples from village milk vendors, Individual milk producers, Small private dairies, milk collection centres and small shopkeepers. He found that the specific gravity, fat percentage, total solids percentage were 1.0304%, 4.39% and 12.923 for milk collected from village milk vendors, 1.0342, 7.01% and 17.77% for milk collected from individual milk producers, 1.0291, 3.68% and 11. 85% for milk collected -from small private dairies, 1.0343, 5.96% and 15.31% for milk collected from milk collection centres and 1.0312, 4.77% and 13.67% for milk collected from small shopkeepers.

Anon (1970) conducted an experiment to determine the composition of milk of 688 samples obtained from 86 farms throughout Crimean province. He found that fat, SNF, TS, and specific gravity were 3.62%, 8.44%, 12.26%, and 1.031 respectively.

Shalichev *et al.* (1972) reviewed the works on composition and properties of, milk of Isker and Sofia Brownswiss cattle. They found that the Specific gravity, total solids, S.N.17, and fat were 1.0335, 9.22%, 4.2% and 3.75% for Iskar and for Brownswiss, the Specific gravity, T.S, S.N.F and fat were 1.033329.126%, 4.97% and 3,577% respectively.

Another experiment was conducted by Uzonyl and Verga (1973) for determining the relevant standard of milk of Hugarian farm and results were 1.029 to 1.033 specific gravity, 3.5% fat, 8.5% SNF.

Arai *et al.* (1976) observed the average composition of raw milk from April 1975 to March 1976 in Miyagi and the compositional values were obtained for fat 3.48 %, T.S 11.65%, S.N.F 9.17% and specific gravity 1.0309. They also observed that the overall mean values for S.N.F.% was 7.98% for other months.

Dehury *et al.* (1977) conducted an experiment to evaluate the physical and chemical quality of market milk at Bhubaneswar of India by collecting milk samples from established dairy farm, local gowalas, local shops milk collection centers and pasteurized milk booths. They found that the specific gravity, fat % and titratable acidity % for samples from local gowalas were 1.03, 4.89 and 0.15; for samples from local shops were 1.004, 3.84, and 0.11; for samples from milk collection centers were 1.03, 4.12 and 0.15 and for samples from pasteurized milk booths were 1.027, 3.11, and 0.14 respectively.

Borges and Rodriques (1978) carried out an experiment to evaluate samples collected from processing plant and market places. They showed that the mean fat and SNF percent were 3.5 and 8.67 in 66 samples of B type milk collected from processing plant and 2.1 and 8.86 percent in 136 samples of C type milk collected from market places.

Chernev *et al.* (1979) observed milk of Bulgarian Simmental, Bulgarian red, Bulgarian brown and Canadian type Holstein. They found the overall mean values and standard deviation of fat were 3.39 ± 0.20%, 3.74 ± 0.32%, 3.68 ± 0.20% and

4.08 ± 0.20% and T.S. were 12.82 ± 0.26%, 12.45 ± 0.35%, 12.24 ± 0.45% and 12.69 ± 0.50% respectively.

Kulkarni *et al.* (1981) carried out an experiment on the acidity development and organoleptic qualities of mixed milk. 180 samples of evening cows' milk were kept over night (15 hours) at room temperature during May and June 1980 in earthen (EW) or metal (MW) containers containing cold water or without any means of cooling (C) They reported that mean temperature of EW and C milk decreased from 35^{0}C before storage to 27^0, 28^0 and 32^{0}C respectively after 15 hours storage and acidity increased from 0.15% before storage to 0.30, 0.34, and 0.51% (< 0.01). On mixing the samples with fresh morning milk (1:1) acidity of EW, MW, and C milk was initially 0.25, .27, and 0.36 % lactic acid, but after 2 hours acidity was 0.29, 0.32 and 0.41%. These mixed samples were not organoleptically acceptable and had positive results in clot on boiling (COB) tests.

Yadav and Sarawat (1982) studied the composition of milk of the market of Varanast town, India. They found that market samples had abnormal colours and taste but normal flavour. The market samples had lower specific gravity and higher acidity.

Jurgens (1983) examined the watering of raw milk in Schleswig- Holstein. The institute of animal health and food quality in Schleswig-Holstein, german federal republic, regularly tests raw milk for watering by measuring the freezing point and titratable acidity. Values for freezing point are corrected when acidity is in the range 7-8 SH, and are disregarded when acidity exceeds 8 SH. Low acidity values (5.3 SH) are in themselves an indicator of possible watering, but normally a reference freezing point of -0.53^0 C is used as the indicator. Milk supplied to 2 dairies over 12 months up to march 1983 had monthly mean freezing point values ranging from -0.528 to -0.53^{0}C, with little seasonal or regional variation. The effect of regular freezing point measurements could be judged from the experience of one dairy, which found that the number of products supplying milk with freezing point values

above -0.525^{0}C decreased from 21 to 1 during this period. In many cases a low level of extraneous water (1-2%) in milk due to farmers being unaware of the presence of residual rinsing water in milking installations, pipelines and tanks.

Islam *et al.* (1984) conducted an experiment to compare the quality of milk between local market milk of Mymensingh Town and- Bangladesh Agriculture University Dairy Farm (BAU), Mymensingh. They reported that the specific gravity and fat percent of milk from Mymensingh town were 1.026 and 3.02 and the same values of milk collected from BAU Dairy Farm were 1.031 and 4.80 respectively.

Gajdusek (1985) carried out an experiment to determine the acid producing activity of raw milk. He took 5723 milk samples from the supply area of certain dairy factory for 2 years. He showed that acid producing activity pattern was similar in both years and lowest value in winter (minimum in February) and peak value in August and September. Amount of acidity produced in milk samples in 1st year and 2nd year were different being influenced by climatic condition.

Simpfenderfer (1988) investigated 335 Holstein milk samples from individual cows. He found that the specific gravity, fat, SNF and TS content of milk were 1.023 to 1.035, 2.00% to 6.00%, 5.41% to 10.74% and 8.21% to 16.74% respectively.

Oldenbroek *et al.* (1988) evaluated the performance of Jersey cows and cows of larger dairy breeds on two complete diets with different roughage contents.38 Jersey cows and a control group composed of 12 Holstein-Friesian, 12 Dutch Friesian [Dutch Black Pied] and 10 Dutch Red and White [Meuse-Rhine-Yssel] cows were fed, after their 3rd calving, a complete diet of roughage or a complete diet of the same roughage with 50% concentrates on a dry matter basis. Body weight, milk yield and feed intake were recorded during the first 39 wk of lactation. A significant breed X diet interaction was found only for fat percentage. For cows fed roughage only, the av. difference between Jerseys and control cows was -936 kVEM (1 kVEM = 6.9 MJ net energy) for energy intake, -2560 kg for milk yield, +2.82 for fat percentage, +0.83 for protein percentage, +3 kg for fat yield, -55 kg

for protein yield, -199 kg for body weight and -57 kg for weight gain. For cows fed concentrates, the av. difference was -748 kVEM for energy intake, -1707 kg for milk yield, +2.38 for fat percentage, +0.77 for protein percentage, +23 kg for fat yield, -26 kg for protein yield, -216 kg for body weight and -27 kg for weight gain. From 1st to 3rd lactation, energy intake and milk yield for the Jerseys and the control increased in those fed concentrates by 25 and 24% resp. vs. 48 and 51% in those fed roughage only. The biological efficiency for milk production (energy in milk divided by net energy in feed) was 57% for Jersey cows fed concentrates, 69% for Jerseys fed roughage only, 56% for the control cows fed concentrates, and 61% for the control cows fed roughage only.

Suchanek *et al.* (1989) observed the composition of milk from cow herds with high milk efficiency. For 19 herds averaging 4450 kg milk for cows in their 1st lactation and 5830 kg for later lactations, milk fat percentage averaged 4.17, 4.01 and 4.21 in Jan.-Mar., June and Sep. resp., protein percentage 3.30, 3.24 and 3.39, non-protein nitrogen 28.0, 29.9 and 28.3 mg/100 g milk, urea 23.8, 22.1 and 28.4 mg/100 g, lactose 4.69, 4.60 and 4.70%, total solids 12.69, 12.47 and 12.77%, SNF 8.69, 8.64 and 8.74%, and ash 0.72, 0.71 and 0.74%. For Czech Pied, Slovakian Pied and Black Pied cows in the above herds, milk fat percentage averaged 4.20, 3.98 and 4.0, protein percentage 3.38, 3.34 and 3.22, non-protein nitrogen 27.4, 13.4 and 39.7 mg/100 ml, urea 26.4, 13.4 and 29.3 mg/100 ml, lactose 4.75, 4.63 and 4.58%, total solids 12.81, 12.58 and 12.42%, SNF 8.71, 8.70 and 8.50%, and ash 0.74, 0.73 and 0.75%. The effect of season on milk composition traits was significant.

Talukder (1989) analyzed some milk samples of indigenous cows from Trisal Upazilla of Mymensingh District. He found that the average percentage of fat, SNF and TS content of indigenous cows' milk were 4.72, 8.61, and 13.33 respectively.

Alam (1989) conducted an experiment on the quality of milk collected from Mymensingh Sadar Upazila and found that the average specific gravity, acidity and fat were 1. 03 2, 0.173 and 4.61% respectively.

Islam (1990) studied the quality of local market milk and market milk collected from co-oparative farmers and found that the milk collected from co-operative farmers were better quality than the local market milk.

Simundic (1991) analysed raw milk samples from the mountainous region of Gorski Kotar, Croatia, Yugoslavia, during the period 1989-90. Yearly average results of 293 milk samples taken in 1989 and 299 samples taken in 1990 are tabulated. Average values for milk fats, TS and SNF were 3.63 and 3.61%, 12.42 and 12.34%, 8.78 and 8.73% for 1989 and 1990 respectively.

Foltys *et al.* (1993) Carried out an experiment where two rations were fed to two groups of Holstein-friesian cows. One diet was balanced and another one contained reduced fibre. It was found that reduced fibre diet decreased the production of milk and fat. Increased content of proteins and decreased content of lactose in milk was observed. Changes in qualitative traits of milk fat and in fatty acids were also observed. Lack of fibre was evident in the increase of saturated and decrease of unsaturated acids from 63.74 to 71.06% and from 36.26 to 28.94% respectively. Decreased fibre diet increased the times of curdling and coagulation of milk. The type of feed ration influenced the creation of VFA in rumen. Lack of fibre in feed ration did not influence significantly the production of total VFA. However, a decrease of acetic acid from 9.15-to 8.38-mmol/100 ml, propionic acid from 2.83 to 2.45 mmol/100 ml and increase of butyric acid from 1.61 to 2.55 mmol/100 ml was evident.

Agabriel *et al.* (1993) studied the factors involved in the chemical composition of milk on farms with a high level of milk production. In this study 76 dairy farms with high-yielding (6200 to 8800 kg/yr) Montbeliarde cows that were given hay-based rations were included in a detailed survey involving the herd and farm structure, the quality of forage, winter and summer feeding practices, and genetic characteristics (breeding value and herd effect for milk production, fat content and protein content). The mean annual fat and protein content. Varied greatly between

farms despite the homogeneity of the farm sample with regard to milk produced, breed and type of winter roughage. Such variability resulted essentially from environmental factors. When farms were classified according to the level of herd effect (fat or protein content), protein content variations were greater in winter and linked to different feed characteristics (hay quality, type of concentrate) and variations in fat content between farm groups were as marked, if not more so, in summer than in winter. These variations were only partly linked to feeding practices that were beneficial or detrimental to fat concn. (Presence of sugarbeet in the ration, concentrate distribution method). No correlation occurred between fat and protein herd effects. It is concluded that these variables may be controlled independently by manipulating environmental factors (especially feeding factors).

Salam (1993) conducted an experiment on the physical, chemical and microbiological qualities of milk produced in Baghabarighat Milk Shed Area and he reported that the means and standard deviations of specific gravity, fat and acidity were 1.02757± 0.001, 5.096 ± 0.389%, and 0.1671 ± 0.009%, respectively.

Senapoty *et al.* (1995) studied on some qualitative data of buffalo raw milk under organized sectors. A comparative study was carried out in Jabalpur, India, between February and April 1988 on raw milk samples produced in organized and non-organized sectors (rural areas of co-operative milk producers union) at the milk distribution point around Jabalpur. Organoleptic evaluation of milk samples showed a significant ($P<0.05$) variation in milk quality. Milk fat, TS, and SNF contents were higher in milk samples from the organized sector compared with that of the non-organized samples sector, but specific gravity of milk samples of both sectors were similar. It was concluded that the milk quality was higher at the farm than on the rural areas.

Mizanur (1995) found that the fat% were 4.28, 4.10, 3.68, 4.95; S.N.F % 7.20, 6.67, 7.04, 7.96; T.S. % 11.48, 10.78, 10.72, 12.91 and the acidity % were 0.150, 0.135,

0.145, 0.159 of Manikganj Chilling Centre, Tangail Chilling Centre, Takerhal pasteurization Plant & Bagbabarighat Dairy Plant.

Rashid *et al.* (1996) stated that mean fat and TS of Bangladesh Agricultural University (BAU) dairy farm milk, its surrounding villages and mini dairy farm in Kawatkhali were 3.721± 0.587, 4.621±0.944, and 3.986±0.428 respectively and 12.62±0.54, 14.32±1.865 and 12.44±0.58 respectively.

Manzoor Quadir (1996) studied that the fat %; SNF % and TS % of Rawtara, Resombari and Briangaru societies at Baghabarighat Milk shed area of Sirajganj were 4.720. 4.547, 4.340; 7.719; 7.788; 7.606 and 12.433, 12.340 & 11.911 respectively.

Feldhofer *et al.* (1998) observed the dry matter and milk protein with regard to breeds and feeding of cows. SNF and protein in milk from cows from 7 individual milk producers in the Lika region. Average milk yield of Friesian cows was higher than that of Brown and Simmental cows. Highest SNF and protein were obtained from Brown cows, while highest milk fat content was obtained from Friesian and Brown cows. Cows fed high-hay diets had the lowest milk yields. Enriched forages increased milk yield and quality. Higher milk yields resulted in lower milk fat, protein and TS, but this was increased by enriched feeding. Average SNF, protein and fat was 8.72-8.93%, 3.38-3.61% and 4.02-4.53%, respectively. Average milk yield was 13.2-21.4 litres/day. SNF and protein were generally similar in milk obtained from morning and evening milking, but higher in evening milk from Brown cows fed 6.5 kg hay, 15 kg silage and 7 kg balanced forages.

Coulon *et al.* (1998) studied on 414-lactation sample to know the effect of pregnancy on the fat and protein contents of milk. 149 lactations of non-pregnant cows managed under identical conditions served as controls. The difference between individual fat or protein contents of each pregnant animal and the mean corresponding values in controls was computed weekly from the week of conception. The effect of pregnancy on fat and protein concentrations began to be

significant from the 20th week of pregnancy, regardless of the week of conception. It was higher in the Friesian breed than in Holstein or Montbeliard cows. In Holstein and Montbeliard cows, the effect of pregnancy was higher in high-producing multiparous cows (+2.8 and +2.9 g/kg for fat and protein concentrations, respectively, at the 29th week of pregnancy) than in primiparous or low-producing multiparous cows (+2.0 and +1.4 g/kg, for fat and protein concentration, respectively). In each of these 3 lactation groups (Friesian, high-producing multiparous Holstein and Montbeliard cows, other Holstein and Montbeliard cows) the following linear model $Y = a(Pw - 18) + b(Pw - 18)2$ was fitted, where Y is the difference between fat or protein concentration of pregnant and non pregnant cows at a given stage (week), Pw is the pregnancy week, and a and b are parameters. This increase in milk fat and protein concentrations in late pregnancy cannot compensate for the concomitant decrease in milk yield, so that fat and protein yield decreased in the same manner as milk yield during pregnancy (-77 g/day and -68 g/day at the 29th week of pregnancy, for fat and protein yield, respectively).

Coulon *et al.* (1998) investigated the effect of extreme walking conditions for dairy cows on milk yield, chemical composition, and somatic cell count. 32 cows (16 Montbeliard and 16 Tarentaise) in mid-lactation were used in a 2 X 2 factorial arrangement between 13 May and 4 July 1996. Cows received first-cutting cocksfoot [*Dactylis glomerata*] hay for ad libitum intake supplemented with a fixed amount of concentrate that was individually adapted to the milk yield of each cow. During the experimental period, 1 group of cows walked 9.6 km/day and the other group remained in the barn. Cows that walked daily ate less hay (-1.3 and -2.1 kg DM/day for Tarentaise and Montbeliard cows, respectively) and yielded less milk (-1.7 and -2.5 kg/day for Tarentaise and Montbeliard cows, respectively) than those that did not. A residual effect of walking on milk yield was observed during the 10 days following the experimental period. For both breeds, fat and protein content were higher (+6.4 and +1.0 g/kg, respectively) for cows that walked. Somatic cell count was also higher for cows that walked (+115 000 cells/ml). This difference

was more marked in cows that were initially infected by a minor or major pathogen (+185 000 cells/ml) than in uninfected cows (+47 000 cells/ml) and on the 1st day of walking, when walking was correlated with increases in pH, bovine serum albumin and immunoglobulin G1 contents of milk (+0.08 unit, +0.16 g/litre and +0.19 g/litre, respectively). Throughout the experimental period, walking induced an increase in body temperature (+1^{0}C) and in plasma non-esterified fatty acids (+0.63 mM/litre). On the 1st day of walking, plasma glucose, lactic acid and cortisol contents were significantly higher for cows that walked (+0.25 g/litre, +0.64 g/litre and +28.8 mg/ml, res pectively).

Kovacs *et al.* (1999) carried out an experiment to know the composition of milk of Hungarian Grey cattle. Data on milk composition and its statistical evaluation are presented, and the results are compared with the values of milk components of cattle of other breeds (Angus, Limousin, Blonde d'Aquitaine and Hungarian Pied). The effect of various production and feeding factors [not specified] are taken into consideration. Milk fat and dry matter content of milk were higher for Hungarian Grey cattle than for other [beef?] cattle, with similar levels to those of the Jersey breed. Average values for composition of dry matter, solids-not-fat, milk fat, milk protein, lactose and ash are given as: 14.21%, 9.27%, 4.94%, 3.46%, 5.08% and 0.73%, respectively.

Steinwidder *et al.* (2000) studied the feeding and animal factors influencing milk urea content of dairy cows. Experimental data (n=1567) were statistically analysed regarding the impact on milk urea content of dairy cows, using nutritional and animal factors. In partial correlation analysis, a weak stochastic correlation between milk urea content and selected parameters for the description of rumen metabolism was found (r=0.3-0.5). The highest correlation to milk urea content was found when digestible crude protein intake was related to energy intake (r=0.5). Regression analysis showed a significant influence of breed and animal, but protein and energy supply of rumen microbes was most important. Additionally, stage of lactation, milk protein yield, supply of utilizable protein in duodenum (nXP) and feed intake

were significant. The lowest residual s.e. was found when using digestible carbohydrate and digestible crude protein intake for describing the rumen nitrogen metabolism in regression analysis. Nevertheless, the relative high residual s.e. of 3.9 mg/100 ml milk urea shows that a remarkably high proportion of variation is caused by factors not considered in the model. This corresponds to numerous data in literature. When milk urea data are interpreted, this inaccuracy should be taken into account.

Mandibaya *et al.* (2000) conducted an experiment in February 1996 to determine the quantity of residual milk suckled by calves for 5 weeks post partum and the composition of the milk immediately before milking in the Nharira-Lancashire smallholder farming areas of Zimbabwe. Seven communal area (CA) and 6 small scale area (SSCA) farmers owning 49 cow-calf pairs of 3 breeds participated in the study. There were no significant differences in the morning milk intake by beef, dairy and dairy x beef (DxB) calves in both CA (mean: 1.39 kg) and SSCA (1.51 kg). Mean pre-weaning growth rates of calves from the 3 breeds were not significantly different in each area (mean: CA = 0.256 and SSCA = 0.343 kg/d). In the CA, the total morning milk yield (milk off-take + calf intake) was 3.5 kg for beef, 4.0 kg for DxB and 5.7 kg for dairy cows; the differences were significant. In the SSCA farms the beef and DxB cows had a lower yield (4.8 and 5.4 kg, respectively) than the dairy cows (7.3 kg). Differences in protein and lactose contents were significant among the 3 breeds in the CA.

Morales *et al.* (2000) observed the effects of fat source and copper on unsaturation of blood and milk triacylglycerol fatty acids in Holstein and Jersey cows. Fatty acid composition of plasma triacylglyceride and milk fat was analysed from Holstein and Jersey cows with control or depleted copper status and fed roasted whole soyabeans or tallow. Conjugated linoleic acid in plasma was higher in Jersey cows. Dietary fat source influenced the proportions of all fatty acids in plasma and in milk, except for conjugated linoleic acid in milk. Feeding soyabeans increased plasma C14:1, C18:0, C18:2,and conjugated linoleic acid, and decreased C14:0,

C16:0, C16:1, and cis- and trans-C18:1 compared with feeding tallow. Low copper diets decreased C18:0 and increased cis- and trans-C18:1, and conjugated linoleic acid in plasma. A fat source x copper status interaction occurred for cis-C18:1 in plasma. Proportions of C4:0 to C14:0 were higher, and cis16:1, cis- and trans-C18:1, and conjugated linoleic acid were lower in milk fat of Jersey compared with Holstein cows. Generally, the effects of copper depletion were less apparent in milk than in plasma. Copper depletion increased C4:0, trans-C18:1, and conjugated linoleic acid, and decreased C16:1 in milk. Feeding whole soyabeans increased C4:0 to C14:0, C18:0, C18:2, and C18:3, and decreased C14:1, C16:0, C16:1, and cis- and trans-C18:1 in milk. Breed x fat interactions occurred for C4:0, C14:1, C16:1, and conjugated linoleic acid in milk. Copper status x fat source interaction occurred for trans-C18:1. The breed x copper status interaction was apparent in milk fat for C16:1 and C18:0 and conjugated linoleic acid in milk. Both C18:0 and trans-C18:1 were desaturated by mammary tissue; however, whereas desaturation of C18:0 was linear, desaturation of trans-C18:1 reached a plateaux that could have been caused by presence of the trans-10 isomer, which is not desaturated and was not separated from trans-11 C18:1 in our analysis. Comparison of the plasma triacylglycerol fatty acid profile with the milk fat profile was useful to interpret separate events of biohydrogenation in the rumen and desaturation by the mammary gland.

Morales *et al.* (2000) studied the effects of breed, dietary fat source, and dietary copper intake as factors known to influence unsaturation of milk fat and its potential for development of spontaneous oxidized flavour in milk. Twelve Holstein and 12 Jersey cows were allotted to 3 blocks with 4 cows of each breed. Cows within breed were allotted randomly within blocks and fed control or copper-depleting diets for 2 months to achieve stable or depleted liver copper stores. Cows then were fed tallow or roasted whole soyabeans in a 2-period switchback (5 weeks per period); during the last week of each period additional vitamin E (2000 IU/day) was added. Copper depletion for 2 months decreased concentrations of copper in liver. Feed intake and

milk yield were influenced only by breed. The proportions of C4:0 to C14:0 and C18:0 in milk fat were higher, whereas C16:1 and cis-C18:1 were lower in Jersey cows. Feeding soyabeans increased C4:0 to C14:0, C18:0, C18:2, and C18:3 in milk, and decreased C14:1, C16:0, C16:1, trans-C18:1, and cis-C18:1. Depleted copper status increased conjugated linoleic acid in milk. Several breed x fat source interactions for individual milk fatty acids occurred. Feeding soyabeans decreased plasma concentrations of copper and zinc, and increased concentrations of alpha-tocopherol in plasma and milk. The concentration of zinc was higher in milk of Jersey cows. Depleted copper status tended to increase copper concentration in plasma and decreased copper in milk. Fat source did not influence plasma copper concentration when status was adequate, but plasma copper concentration was higher when tallow was fed to cows with depleted copper status. Supplementing vitamin E increased concentration of alpha-tocopherol in plasma and milk and decreased concentration of zinc in milk. Factors influencing the potential for oxidized flavour development in milk can be manipulated by changing the diet of the cow.

Stene *et al.* (2002) investigated the conjugated linoleic acid (CLA) content of milk from cows in two different production systems. Two systems compared were a herd of 19 Norwegian Red cows kept under organic farming conditions, with spring calving and high milk production during the grazing season (May to September), and a herd of 48 cows of the same breed kept under conventional conditions, with calving from September to December. Milk samples were taken monthly from individual cows in each group, from April to December in the organic group and from October to December in the conventional group. Analysis of the samples showed that the average content of conjugated linoleic acid (CLA) was higher in the organic group. This was attributed to the fact that, because of early calving, the animals spent most of their lactating period at pasture, whereas the high level of concentrates fed to the conventional group that were housed during lactation

resulted in a significantly higher ratio between omega-6 and omega-3 fatty acids in their milk.

Piccioli *et al.* (2003) carried out an experiment on the milk yield and changes in its composition, as well as those of some blood metabolites and hormones in dairy cows (n=6) of low-average genetic merit, when intrajugularly infused with a short load of glucose or amino acids or a mixture of these was investigated. Infusion of glucose, regardless of stage of lactation, resulted in increased milk yield significantly. Although fat content of milk remained almost constant in all stages of lactation, it was always reduced when glucose was infused, either alone or with amino acids. The reduction, however, was more pronounced in animals at the third stage of lactation. Infusion of amino acids always resulted in slightly reduced fat contents. The results indicate that the quick raise of blood substrates can modify milk yield and its composition. Glucose substantially increases lactose and milk yield, with a subsequent reduction in protein and fat contents (dilution effect).

Bortolozzo *et al.* (2003) observed the effect of pasture and soybean supplementation on fatty acid profile and CLA content in dairy cow milk. Eighteen Friesian cows (primiparous and multiparous) in mid-lactation (147ñ49 days) with a milk yield of 33ñ6 kg/day were fed ad libitum, after an adaptation period of 14 days, with one of three dietary treatments over 3 periods lasting 4 weeks each. The treatments were: TS (mixed diet + 2.6 kg/day toasted soyabean), RS (mixed diet + 2.6 kg/day raw soyabean), and PRS (pasture+concentrate+2.6 kg/day raw soyabean). The mixed diet contained chopped corn (24.2%), barley meal (20.5%), lucerne hay (20.5%), meadow hay (17.9%), and dehydrated lucerne hay (16.8%). The PRS group received a fixed amount of chopped maize (3.5 kg/day), dehydrated lucerne hay (2.4 kg/day), and raw soyabean in addition to pasture. Dry matter intake was similar for TS and RS, and higher than PRS. Milk yield decreased significantly $P < 0.05$) in grazing cows compared with the other groups, but fat and protein content were unaffected by dietary treatment. The highest milk urea was recorded for PRS. A significantly lower value of short-medium chain fatty acids was recorded for

grazing cows. The results indicate that the fatty acid profile and CLA content in milk is mainly affected by pasture than by the sources of soyabean supplementation, and there are no differences between toasted and raw soyabeans supplemented in mixed diets.

Wijesundera *et al.* (2003) carried an experiment to determine the effects of cereal grain and fibre (hay or straw) supplements on the fatty acid composition of milk fat of grazing dairy cows in early lactation. In both experiments, grain supplements significantly increased ($P < 0.05$) the proportion of the endogenously synthesized 10:0-16:0 fatty acids. Of the C18 acids, the proportion of 18:0 and 18:3 was significantly decreased ($P < 0.05$) by grain supplementation, while that of 18:2 was significantly increased ($P < 0.05$). Irrespective of diet, 18:1 trans-11 was the most dominant trans 18:1 isomer in milk fat. In the first experiment, the proportions of the 18:1 trans-11 isomer and conjugated linoleic acid (CLA, 18:2 cis-9, trans-11) were highest for the pasture-only diets, and significantly ($P < 0.05$) decreased with grain supplementation. The opposite result was observed in the second experiment, conducted in a different dairy region, suggesting that factors such as the quality of pasture on offer and the physiological state of the cow could affect the content of CLA and trans fatty acids in milk fat. In both experiments, there was a significant positive linear relationship between CLA and 18:1 trans-11. Fibre supplements had little effect on the fatty acid composition of the milk.

Sterna *et al.* (2003) observed the comparison of fatty acids and cholesterol content in the milk of Latvian cows. Milk samples were obtained from Latvian Brown and Black and White cows fed the same diet from a farm in Riga, Latvia [date not given]. Fatty acid composition and cholesterol content were analysed by gas chromatography. Differences in the fatty acid composition, cholesterol and fat content between the breeds were observed. Latvian Brown cows showed higher milk fat content (4.88ñ0.68% vs. 4.25ñ0.13%) and cholesterol level (18.63ñ3.58 mg/dl vs. 16.25ñ1.20 mg/dl) compared to Black and White cows. Milk of Latvian Brown had significantly higher fat and protein content, while Black and White cows

had a higher milk yield. The content of saturated fatty acids was different between the two breeds. The amount of myristic acid, which affects the cholesterol content in plasma, was the same for both breeds of cow at 0.37 g/100 g.

Kaewkamchan *et al.* (2003) conducted an experiment to investigate the factors affecting yield and composition of milk produced from dairy cow raised under hot and humid environment of Thailand. Forty dairy farms were randomly selected and divided into two groups of twenty each according to NRC's (1988) nutrient requirements (standard and understandard feeding). The farms were monitored monthly for one year. The dairy breeds employed, feed management practices, milk yield, and milk composition were determined. It was observed that milk yield, fat, protein, and SNF from cows receiving standard feeding were 12.70, 5.72, 4.66 and 3.18% higher ($P < 0.01$) than those from the group fed diet below the recommended nutrient standards. With increasing Holstein-Friesian (HF) bloods, milk yield increased, while milk protein and SNF decreased ($P < 0.01$). However, a decline ($P < 0.01$) in milk fat was observed in cows receiving standard nutrient requirements but with higher HF blood levels. When compared to winter and summer, cows during the rainy season, produced the lowest ($P < 0.01$) milk yield with highest fat, protein, and SNF contents.

Brien, *et al.* (2003) observed the effect of milking frequency on yield, composition and processing quality of milk. The objectives of the study was to establish if once daily milking (ODM) or omitting one milking weekly are feasible labour saving options in Irish herds, given expected changes in cow performance and milk quality (Irish Republic). A total of 72 cows were studied. Once daily milking reduced milk yield by 29% and did not adversely affect the processability of milk.

Lock *et al.* (2003) observed the Seasonal variation in milk conjugated linoleic acid and DELTA9-desaturase activity in dairy cows. Main objective of this work was to study changes in the fatty acid profile of cows' milk throughout the year with

particular emphasis on cis-9, trans-11 conjugated linoleic acid (CLA), a proven anticarcinogen, found predominantly in milk and meat from ruminants. During the winter months, a total mixed ration of grass and maize silages, brewers grains, cereals, soya and dairy concentrates was fed. Through the summer months, fresh grass was fed, with increasing levels of buffer feeding given as the summer progressed. The CLA content of milk in May, June and July was significantly higher ($P<0.05$) than all other months, averaging 1.50 g CLA/100 g FAME compared with a mean of 0.77 g/100 g for the other months. DELTA9-Desaturase activity was also greater in the summer. Milk fat produced during the summer contained significantly ($P<0.05$) greater amounts of short-chain fatty acids at the expense of medium-chain fatty acids indicating that fresh grass may alter the pattern of fatty acids produced de novo in the mammary gland. Results suggest that fresh grass promotes the synthesis of CLA in the dairy cow through an increase in DELTA9-desaturase activity in the mammary gland and possibly other unknown factors.

Gonzalez *et al.* (2004) studied the evaluation of milk quality in different months of the year at the Pelotas dairy basin. The effect of months of the year on milk production and quality was estimated in 10 dairy production units classified as specialized (S), partially specialized (PS) and not specialized (NS), which were visited for 11 months within the year [date not given]. Bulk tank milk was sampled to determine the physical and chemical characteristics and somatic cell count (SCC), mastitis percentage and milk production (litres/cow/day). Samples of feeds and water used for drinking and cleaning were also collected. There were no significant differences between months for milk production, crioscopy, percentages of fat, total solids, non protein nitrogen and SCC, but true protein percentages were higher in October and November, and casein showed higher values in October, November, March and April. Total solids were higher in December. Acidity varied in the same way as mastitis percentage, being higher in November and May. Negative relationship between milk production and percentage of fat, mastitis and

somatic cell count were detected, while a positive relationship was observed between milk production and lactose percentage. Milk obtained during the year showed differences between months for protein fraction, acidity, solids non fat and percentage of mastitis. Water quality did not change among collection months and was acceptable for drinking and cleaning.

Hanus *et al.* (2004) observed the effects of later summer pasture on production, quality, composition and technological properties of raw dairy cow milk in model herd in the Czech Republic. The aim of this paper was to describe the effects of pasture on milk composition and properties under Czech Republic conditions. Four groups of samples were created in terms of lactation stage and lactation number: I, summer outdoor grass pasture with a small part of preserved rough fodder addition; II, the same dairy cow herd (I) during winter indoor feeding; III, other dairy cow herds fed by total mixed ration (TMR) during summer; IV, other dairy cow herds fed by TMR during the whole year. The obtained results revealed that some milk parameters (somatic cell count (PSB), acetone concentration (Ac) and all microbiological data) were statistically evaluated due to their unequal frequency distribution. The solids not fat (STP) contents (I>II, III and IV) were regularly influenced by dairy cow pasture feeding. Similar situation was observed in milk urea when compared with nongrazing dairy cows. Higher individual protein fractions (crude protein (HB), true protein (CB) and casein content (KAS) at 3.61, 3.36, 2.79%, respectively, and I>II, III and IV) were observed in grazing dairy cows. The higher whey protein (SB) was observed in pasture conditions as well. A higher nonprotien nitrogen milk content was also observed in pasture conditions. The effect of grazing was also observed in Ac concentration. The frequency of mastitis pathogen (Streptococcus agalactiae (SAG) and Staphylococcus aureus (SAU)) and thermoresistant microorganisms (TRM) in milk were a little lower in pasture conditions. TRM was also lower in the faeces of grazing cows. It is suggested that there is better milk hygiene during pasture conditions and that

grazing has some positive effects on milk production. The pasture, therefore, is underestimated in dairy cow nourishment and feeding.

Chilliard *et al.* (2004) studied the dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. The effects of dietary factors on bovine and caprine milk fatty acid composition, as well as the regulation of cow and goat mammary lipid secretion was studied. Special attention was given to fatty acids that could play a role for human health, such as saturated fatty acids, oleic acid, n-6- or n-3-C18 to C22 polyunsaturated fatty acids, trans isomers of C18:1 and C18:2, and isomers of conjugated linoleic acid (CLA). The main dietary factors taken into account are the nature of forages, including pasture, the forage:concentrate ratio and diet starch content, and the supplementation of dairy rations with crude or processed vegetable oils or oilseeds and vitamin E. Particular emphasis is given to studies on interactions between these dietary factors, which show that there is a considerable plasticity of ruminant milk fatty acid composition. Despite the existence of several studies on the effects of dietary factors on the sensorial quality of milk and dairy products, there is a need to evaluate more deeply how the different feeding strategies could change the nutritional, sensorial and technological aspects of milk fat quality.

Walker *et al.* (2004) studied the effects of nutrition and management on the production and composition of milk fat and protein. The composition and functional properties of cow's milk are of considerable importance to the dairy farmer, manufacturer and consumer. Broadly, there are 3 options for altering the composition and/or functional properties of milk: cow nutrition and management, cow genetics and dairy manufacturing technologies. The effects of nutrition and management on the composition and production of milk fat and protein, and the relevance of these effects to the feeding systems used in the Australian dairy industry was studied in this experiment. Dairy cows on herbage-based diets derive fatty acids for milk fat synthesis from the diet/rumen microorganisms (400-450 g/kg), from adipose tissues (<100 g/kg) and from de novo synthesis in the

mammary gland (about 500 g/kg). However, the relative contributions of these sources of fatty acids to milk fat production are highly dependent upon feed intake, diet composition and stage of lactation. Feed intake, the amount of starch relative to fibre, the amount and composition of long chain fatty acids in the diet and energy balance are particularly important. Significant differences in these factors exist between pasture-based dairy production systems and those based on total mixed ration, leading to differences in milk fat composition between the two. High intakes of starch are associated with higher levels of de novo synthesis of fat in the mammary gland, resulting in milk fat with a higher concentration of saturated fatty acids. In contrast, higher intakes of polyunsaturated fatty acids from pasture and/or lipid supplements result in higher concentrations of unsaturated fatty acids, particularly oleate, trans-vaccenate and conjugated linoleic acid (CLA) in milk fat. A decline in milk fat concentration associated with increased feeding with starch-based concentrates can be attributed to changes in the ratios of lipogenic to glucogenic volatile fatty acids produced in the rumen. Milk fat depression, however, is likely the result of increased rates of production of long chain fatty acids containing a trans-10 double bond in the rumen, in particular trans-10 18:1 and trans-10-cis-12 18:2 in response to diets that contain a high concentration of polyunsaturated fatty acids and/or starch. Low rumen fluid pH can also be a factor. The concentration and composition of protein in milk were largely unresponsive to variation in nutrition and management. Exceptions to this are the effects of very low intakes of metabolizable energy (ME) and/or metabolizable protein (MP) on the concentration of total protein in milk, and the effects of feeding with supplements that contain organic Se on the concentration of Se, as selenoprotein, in milk. In general, the first limitation for the synthesis of milk protein in Australian dairy production systems is availability of ME since pasture usually provides an excess of MP. However, low concentrations of protein in milk produced in Queensland and Western Australia, associated with seasonal variations in the nutritional value of herbage, may be a response to low intakes of both ME and MP. Stage of lactation is

important in determining milk protein concentration, but has little influence on protein composition. The exception to this is in very late lactation where stage of lactation and low ME intake can interact to reduce the casein fraction and increase the whey fraction in milk and, consequently, reduce the yield of cheese per unit of milk. Milk and dairy products could also provide significant amounts of (Se) as selenoproteins, in human diets. Feeding organic selenoproteins (Se) supplements to dairy cows grazing pastures that are low in selenoproteins (Se) may also benefit cow health. Research into targeted feeding strategies that make use of feed supplements including oil seeds, vegetable and fish oils and organic Se supplements would increase the management options available to dairy farmers for the production of milks that differ in their composition. Given appropriate market signals, milk could be produced with lower concentrations of fat or higher levels of unsaturated fats, including CLA, and/or high concentrations of selenoproteins. This has the potential to allow the farmer to find a higher value market for milk and improve the competitiveness of the dairy manufacturer by enabling better matching of the supply of dairy products to the demands of the market.

Jonkus *et al.* (2004) carried out an experiment to see the daily milk productivity change in dairy cows. Research on the fluctuations in cow milk productivity traits was carried out in July and August 2001 and 2002 for a period of 30 days each year. Latvian Brown milking cows, 74 and 66 respectively, reared by one person, were included in the trial group. Coefficients of variation were calculated for each cow for all the studied productivity traits to elicit dynamics of milk productivity traits. It was shown that the dynamics of cow daily milk productivity was higher in 2002. In both trial years, the greatest values of coefficients of variation were obtained for somatic cell count, 16.83% and 37.01% on average per group of cows. Lactose content in milk was the most stable milk productivity trait. The observed significant variability was from 1.43-3.37%. The average variability in milk yield, fat and protein content in milk was 9.77, 10.94 and 8.76%, respectively, in 2002. Physiological factors, such as cow age at lactations and lactation phase, might be

the factors which could significantly affect milk productivity dynamics. Feeds used as supplements in the cow diets and concentrated feeds significantly affected dynamics of fat, protein and lactose contents in milk. Analysis of the dynamics of the milk yield, fat and protein contents in milk in different cow lactation phases showed that these traits could significantly change the following day. During the second trial day, cows of the 1st phase lactation showed significant changes in average milk yield, fat and protein contents in milk by 6, 10 and 6%, respectively, compared to the first trial day.

Yeasmin *et al.* (2004) observed the effect of urea molasses multi-nutrient blocks supplementation of dairy cows fed rice straw and green grasses on milk yield, composition, live weight gain of cows and calves and feed intake. The effects of supplementation with urea molasses multinutrient blocks (UMMB) of dairy cows fed rice straw and green grasses on milk yield, composition, liveweight gain of cows and calves and feed intake were studied under village conditions in Bangladesh. The cows were offered 250 g UMMB per cow per day. The animals were divided into 2 groups and randomly assigned to 2 dietary treatments. The control group (A) received a diet containing rice straw, green grasses, wheat bran, rice polish and mustard oil cake, while the supplemented group (B) received UMMB in addition to the diet given to the control group. It was shown that supplementation of UMMB to cows also receiving straw-based diets increased milk production from 2.86 to 4.43 litres/day ($P<0.01$) and liveweight of calves from 20.29 to 25.57 kg ($P<0.05$). However, supplementation did not significantly increase the liveweight gain, body condition score, milk composition and intake of cows. This increase in milk yield was mainly explained by increased intakes of energy and nitrogen. UMMB is recommended as a strategic supplement in lactating dairy cows fed on low quality roughages or crop residues.

Pirlo *et al.* (2004) conducted an experiment where a total of 105 Italian Friesian cows, with 69 heifers, were allotted to two milking systems (automatic milking system (AMS) or milking parlour (MP)). Milk was stored at 4-5 degrees C. From the experimental herd, 38 heifers were selected for additional analyses concerning metabolism and milk characteristics. 21 cows were milked using MP and 17 with AMS. In addition, the entire bulk milk of the two experimental groups was used for the production of 30 Grana Padano cheeses. AMS cows were milked 2.56 times a day on average. No significant difference was observed in overall milk production between AMS and MP. Slight differences between MP and AMS were observed for some blood parameters at the beginning of lactation and for cortisol since the first day after introduction into AMS, suggesting a light but prolonged stress-like situation. Milk fat was 3.61 and 3.33% ($P<0.05$) for MP and AMS, respectively. However, no difference was observed for milk protein and lactose content. SCC was higher in AMS than in MP, pH was 6.706 and 6.759 ($P=0.002$), FFA (meq/100 g of fat) was 0.531 and 0.700 ($P=0.001$) and titratable acidity (degrees SH/100 ml) was 7.143 and 6.759 ($P=0.002$) for MP and AMS, respectively. No significant differences were found for other milk characteristics. Cheese of MP and AMS groups had very similar characteristics.

Back *et al.* (2004) conducted an experiment to investigate the effects of a commercial feed containing conjugated linoleic acid on the production of milk components and the value of milk This study was to evaluate changes in cow productivity and milk value from feeding a rumen-protected form of CLA to pasture-fed dairy cows in early lactation. 30 New Zealand Friesian dairy cows were randomly assigned at calving to either 0 g (control) or 120 g/d of a commercial rumen protected feed containing CLA from 4 days post-calving for 16 weeks. Milk yield and composition data was collected weekly. A simulation model was used to calculate yield of dairy products based on accumulated yields of milk and its components per cow during the experimental period. Feeding CLA reduced ($P<0.001$) fat concentration (3.1% vs. 4.2%) and increased ($P<0.05$) milk yield

(2688 kg vs. 2423 kg), and protein yield (89.9 kg vs. 81.2 kg). The effect of CLA on milk fat concentration was reflected in the yield of dairy products and milk value. Milk from cows fed CLA had less ($P<0.01$) excess of fat sold as butter (48.4 vs. 69.4 kg butter). The value of milk ($/kg MS) was higher ($P<0.001$) from cows fed CLA than from control cows at 2002/03 prices ($3.87 vs. $3.66) and when butter price was low ($3.56 vs. $3.24).

Politis *et al.* (2004) conducted an exprement to determine the effect of vitamin E supplementation on immune parameters, milk composition and milk quality on fifty-six Holstein dairy cows from a commercial dairy herd in the Northern part of Greece. Cows were assigned to one of two experimental groups: control (no vitamin E supplementation) and vitamin E supplementation. Supplementation of vitamin E started 4 weeks prior to and continued up to 12 weeks after parturition. Supplementation included daily oral administration of vitamin E at 3000 i.u./cow prepartum and was reduced to 1000 i.u./cow post partum. Blood samples were collected weekly for 8 weeks starting 4 weeks before parturition, neutrophils were isolated and the following parameters were determined in neutrophils activated by phorbol myristate acetate: total cell-associated and membrane-bound urokinase plasminogen activator (u-PA) activity and superoxide production. Milk samples were collected weekly and fat, protein, lactose, somatic cell count (SCC), plasmin and plasminogen-derived activity were determined. Activated neutrophils isolated from cows that received supplemental vitamin E had higher ($P<0.01$) total and membrane-bound u-PA activities during the first 3 weeks after parturition and higher ($P<0.01$) superoxide production during week 1 prepartum and week 1 post partum compared with the corresponding values of activated neutrophils isolated from control cows. Vitamin E supplementation had no effect ($P=0.28$) on plasminogen-derived activity in milk. Milk obtained from cows that received supplemental vitamin E had SCC lower by 25% ($P<0.05$) and plasmin lower by 30% ($P<0.01$) than corresponding values in milk obtained from control cows. The reduction in plasmin as a result of vitamin E supplementation is very beneficial to

the dairy industry because plasmin reduces the cheese-yielding capacity of milk, affects the coagulating properties of milk and its overall ability to withstand processing during cheesemaking. In conclusion, vitamin E supplementation had positive effects on the function of bovine neutrophils and milk quality in a commercial dairy herd.

Juozaitiene *et al.* (2004) conducted an experiment to determine the relationship of somatic cell count with milk yield and composition in the herds (1813) of Black and White cattle and the influence of environmental and genetic factors on the quality of milk according to somatic cell count. The data included milk yield, composition and somatic cell count (SCC) from test day records of 6001 cows (of the first three lactations) between 1998 and 2002. Analysis indicated a significant influence of the herd on SCC. The SCC in the milk of Black and White cattle population had a tendency to increase with the increase of cow number in a herd. The number was lowest in small farms of 1 to 5 cows (293.9 103/cm3) and highest in farms of 101 to 200 cows (425.1 103/cm3). The lowest SCC was noticed during summer–288 103/cm3, which was 139 103/cm3 lower than during winter or autumn. The highest quantity of milk was obtained during summer ($P<0.001$). The lactation had a great influence ($p<0.001$) on the traits of SCC, milk yield and composition. With the increase of lactation, the SCC increased from 247.3 to 392.4 103/cm3. The stage of lactation also influenced ($p<0.001$) the SCC in milk. By performing the investigations according to the stages of lactation, we determined the increase of SCC in milk during lactation I–from 239 to 334, during lactation II - from 302 to 453, during lactation III–from 324 to 561 103/cm3. The research showed a negative impact of SCC on the milk yield and composition of Black and White cows. The correlation between the SCC log2 and milk yield and its composition was significantly negative (with milk yield $r = -0.34–0.39$, lactose – $r=-0.38–0.44$, milk fat – $r = -0.03–0.04$ and protein in % – $r = -0.01–0.02$). The multivariate mixed animal model was used to estimate heritabilities. The heritability for log2

SCC was low – 0.07–0.13. Somatic cell count in milk must be considered as an indirect method of improving mastitis resistance in herds of Black and White cattle.

Han *et al.* (2004) observed the environmental and physiological factors affecting milk yield and compositions of Holstein–Friesian cows in Korea. Factors associated with milk urea nitrogen on milk yield and milk composition were investigated in this study with regards to feeding management and physiological status of lactating dairy cows. Data for 3 years (1999–2002) were collected from 129 645 cows by Korean Agricultural Cooperatives Federation in the Korea Republic. The objectives of this study were to describe the relationships between milk urea concentrations and seasonal factors, cow factors and production of milk, milk fat, protein and somatic cell score (SCS). Milk urea was highest in summer and also showed a nonlinear association with milk yield. Milk yield was higher at milk urea concentrations of 21-24 mg/dl; however, it decreased at milk urea concentrations higher than 24 mg/dl. Milk urea was higher with increased parities of cows and in particular, at 3 to 4 parities. There was a negative association between milk urea and SCS in milk. SCS in milk was lowest at milk urea concentrations of 21-24 mg/dl. Milk fat and milk protein were greatly affected by days in milk (DIM), year of birth, season and milk urea concentrations, respectively. While milk urea increased in summer, milk fat and protein decreased. Milk protein decreased according to longer days in milk. With regard to the influences of parity, milk protein was negatively correlated to milk urea in all lactations; however, the extent of the decrease of milk protein at high concentrations of milk urea was bigger during the third lactation. The balanced supply of energy and protein to the dairy cows might have greatly affected the urea concentrations and protein content of milk.

Rashida *et al.* (2004) conducted an experiment to study the comparative analysis of quality of milk collected from buffalo, cow, goat and sheep of Rawalpindi/Islamabad Region in Pakistan. A comparative analysis of cow, goat, buffalo and ewe milk was conducted. All animals came from the Rawalpindi/Islamabad Region in Pakistan. A total of 40 fresh milk samples (10

from each animal species) were collected and subjected to physical and chemical analysis. The highest lactometer reading and specific gravity values were observed in cow milk. The maximum pH and titratable acidity values were observed in cow and ewe milk, respectively. The minimum pH and titratable acidity values were found in ewe and cow milk samples, respectively. The lowest value of total titratable acidity was observed in buffalo milk (0.11%), whereas the highest value was found in ewe milk (0.18%). Insignificant differences in fat percentage were observed among buffalo, cow and goat milk, whereas significant differences were observed between ewe milk and the other 2 species' milk. Solids not fat in cow and goat milk were insignificantly different. However, significant differences were observed in ewe and buffalo milk compared to the milk from the 3 species ($P<0.05$). Although ewe milk showed the highest value of total solids (8.96%) and buffalo milk showed the lowest value (5.25%), the differences were insignificant. The highest and lowest protein contents were shown by ewe and goat milk, respectively. The protein contents of cow and buffalo milk (5.23 and 3.87%) were significantly different from each other and from ewe milk. The percentages of total nitrogen and nonprotein nitrogen were significantly different among the species. Lactose content of goat milk was significantly different from that of other species' milk. The ash content of ewe milk was significantly different from that of milk coming from the other species. Cow milk was found to be the best in quality among the milk samples (buffalo, goat and sheep).

Wiewiora *et al.* (2004) conducted an experiment to determine the effect of dietary iodine and selenium and their interaction on cow performance, milk composition and characteristics, iodine and selenium concentration in blood and milk, and concentration of thyroid hormones in blood. The study was carried out in a randomized block design with 32 Red–and–White cows assigned to four groups of 8 cows each. The 126–day experiment consisted of three 42–day periods. The dietary ration contained pasture grass, fresh brewer's grain, a feed mixture and a mineral mixture differing in iodine and selenium concentrations. The dietary

concentration averaged 1.69 and 2.99 mg/kg DM for iodine and 0.32 and 0.54 mg/kg DM for selenium. The effect of iodine intake was not significant on the milk yield, fat and protein content of milk, and milk density. Higher selenium doses significantly increased the milk yield and the protein content of milk. Cow performance during the experiment averaged 20.85+or-0.72 kg/day, with 3.82+or-0.07% fat content and 3.26+or-0.4% protein content. Milk acidity was 6.53+or-0.10 degrees SH and milk density 1.0300+or-0.0002 g/cm3. The iodine concentration in milk for both dietary iodine concentrations was 92.92 and 132.65 micro g/1000 ml, and that of selenium 6.14 and 8.94 micro g/1000 ml, respectively. 5.2 to 7.5% iodine and 1.9 to 2.7% selenium were secreted in milk in relation to their intake. The higher iodine dose significantly increased magnesium concentration in blood plasma and sodium and zinc concentration in milk. The higher selenium doses significantly increased the concentration of calcium in milk. There was no significant interaction of iodine and selenium in their effect on the min0eral components of cows' blood and milk. The higher iodine doses significantly increased the level of triglycerides, total cholesterol and LDL and HDL fractions in blood plasma. No significant interaction of iodine and selenium in their effect on metabolic indicators of glucose, protein and fat was found.

Hanus *et al.* (2004) studied the transport and treatment conditions of routine milk samples as important factors of the analytical result quality. Questionnaires were sent to different countries regarding the milk treatment and transport practices important in quality controls in order to help improve milk quality in the Czech Republic. Information from 31 systems in different states, countries and regions from 5 continents for individual and bulk milk sample preservation, transport and storage are presented. The results were also sent back to the respondents. For individual milk samples, there was a trend for medium (30-40 ml) milk sample volumes. Most samples were preserved in bronopol and were transported in special carriages. Efforts were made to decrease transport periods and to transport milk samples in special vehicles with refrigerated compartments at decreased

temperatures. For bulk milk samples, there was a trend for large (30-40 and >40 ml) milk sample volumes. Most samples were not preserved and were also transported in special containers. Most samples brought to the laboratory were less than 1.5 days old and were transported in vehicles with refrigerated compartments at temperatures of <12 degrees C.

2.3 Microbiological parameters of milk

Ikonomov *et al.* (1956) studied the hygiene of milk production on a Bulgarian cattle breeding farm. They reported that the total bacterial counts ranged from 170000 to 9,000,000 per ml of milk depending on milking techniques and cleanliness. The number of bacteria in aseptically drawn milk was 100-92,000 per ml, but infection occurred subsequently from the skin of animals, milkers hands, cowshed and milking utensils.

Pal and Singha (1965) studied the bacteriological quality of raw market milk collected from confectioners, organised dairies and importers (agencies which collected milk from villages, producers distributors or collector distributors) in Ludhiana city. They reported that on the basis of plate counts milk obtained from confectioners and importers were extremely poor, containing 10 million per ml of bacteria respectively.

Marutiram and Singh (1969) studied the bacteriological quality of raw milk collected from cooperative union, dairy institute and military dairy farm of Allahabad in India. They found that the methylene blue reduction time was 36 min, 102min, and 393 min and total bacterial counts were 9.17, 1.74, and 0.02 million/ml respectively.

Khan (1969) reported that milk obtained under aseptic condition contain small number of bacteria ranging from 300 to 2000/ml and samples collected under poor sanitary condition had higher total bacterial count ranging from 10000 to 100000/ml.

Rahman (1974) studied the bacteriological density of milk for individual cows milk sold by village vendors, town market milk, milk from hotels, tea stall and sweet meat shops. The results obtained were 2.2×103^{-3}-3.3×10^{3}, 6.5×10^{5}, 7.4×10^{7}, 1.05×10^{7}, 1.8×10^{7}, and 6.5×10^{7} viable bacteria per ml respectively.

Garg *et al.* (1977) studied on pathogenic bacterial flora of raw milk. Standard plate count in raw market buffaloes' and cows' milk samples respectively was (million/ml) 0.1-32, and 0.54-40 in winter, and 0.03-13 and 0.4-200 in summer.

Singh and Ranganathn (1978) analysed 30 samples of pasteurized cows milk and obtained 15 samples positive for *Escherichia coli* in which the range varied from 0-4500 per ml. In another 27 samples of pasteurized Buffalo milk were tested in which 15 samples revealed coliforms. The range was between 0-10,000 per ml.

Golubeva (1981) was studies the bacterial contamination of cows' milk. Cows were milked in to portable tanks after the udder had been washed with warm water and wiped with a cloth. Samples were taken immediately after milking and after storage of milk for 12-24 hour at 12- 14^{0}C for examination. The bacterial counts in unstored and stored milk sample ranged, respectively from 100,000 to 5.5 million and from 400,000 to 23.5 million/ ml.

Urbach and Milne (1987) stored pasteurized milk at 4, 7 and 10 deg. C until it was no longer palatable. The microbiological quality of the milk and the level of volatiles in the milk were determined at intervals. A marked increase in the level of ethanol in the milk corresponded with the onset of off flavour development and with a standard plate count of the order of 106-107 c.f.u. per ml. The increase in ethanol was paralleled by an increase in acetaldehyde, although this was an order of magnitude smaller. An increase, in propan 2-01, together with a corresponding decrease in acetone, seemed to be dependent on the contaminating strain.

Misra and Kulla (1989) analysed 25 pasteurized milk samples collected from dairy plants. They demonstrated that mean standard plate and coliform count per ml was 1,53,00 and 120 respectively in the samples. The authors found the psychrophilic

bacteria as the main contaminants with counts ranging from 20×10^2 to 30×10^5 per ml.

Crolmie and Dommett (1989) stated the relationship between bacterial counts and acceptability of refrigerated pasturized milk. They stored pasteurized milk at 4^0C and 7^0C for up to 21 days. During storage, the samples were subjected to sensory evaluation, microbiological analysis and chemical analysis. The parameters that gave the highest correlations with the flvour-acceptability score were standard plate count (r = -0.70) for milk stored at 70c and gram negative count (r = -0.58) for milk stored at 4^0c. These results showed poor relationships between microbiological counts and flavour acceptability of pasteprized milks which confirmed similar conclusions in overseas studies.

Cox and Macrae (1989) examined the quality of pasteurized goat milk based on advisory standards employed for cow's milk (i.e. 50,000 for farm raw and unpasteurized milk and 1,50,000 cfu per ml for factory milk). They found that out 23 (17 per cent) pasteurized milk samples, 4 samples (17 per cent) were below these standards. A great increase in some groups of microbial population during refrigerated storage suggested the occurrence of significant initial contamination of milk with psychrotrophs.

Alam *et al.* (1989) studied on milk quality produced traditionally and hygienically under Mymensingh Sadar Upazilla. The mean bacterial count and there standard deviation obtained by DMC in milk of traditional and hygienic condition were $6.25\times 10^6\pm 1.6\ x10^6$ and $5.5\ x\ 10^6\pm 1.61\ x10^6$ bacteria/ml. the difference between bacterial count of two types of milk samples was highly significant ($P<0.01$). From this result it is evident that due to improving the hygienic condition during milking a large number of microbial populations was reduced in milk.

Pann (1982) studied on the total bacteria in raw milk. The results for total bacterial counts obtained for milk supplied to a hard cheese factory in balzhurg, showed that

91 per cent of the 2235 samples contained <5,00,000 bacteria per ml and 8 percent up to 2500000 per ml; 94.8 percent of the samples were graded quality calls one.

Harry *et al.* (1982) examined 185 samples of pasteurized milk for bacterial viable counts. These samples were kept at 7^0c for 10 days and at 18^0c for 45 hours. An additional test, to determine catalase-positive microorganisms were enumerated at 180C for 45 hours. The author suggested that incubation at 18°c for 45 hours exhibited a reliable estimated number of psychrotrophs in pasteurized milk.

Reichart (1982) studied the quality of raw milk and reported that the raw milk supplied to dairy plant from high quality diary farm contained 0.3 million/ml bacteria.

Lee *et al.* (1983) conducted an experiment in Seoul area of Korea and found that the bacterial count in raw milk ranged from 4×10^6 to 2.7×10^7 per ml.

Galton *et al.* (1984) showed that wet surface of udder and teats gave a higher standard plate count than wet teats only. Physical action of cleaning teats with dry towel reduced bacterial counts compared with preparation wetting udder surface and teats. Physical manipulation of teats during cleaning was essential for lower sediment in milk.

Mahari and Gashe (1990) observed the microflora of raw and pasteurized milk and source of contamination at the processing plant. Lowest count for raw milk was 4×107 and highest 1×109 cfu per ml as it left the pasteurizing unit. But the population increased 2 to 4 fold as a result of subsequent contamination. The total counts in raw, milk, psychriphilic, thermoduric and thermophilic organisms were 98.1, 1.4 and 0.5 percent respectively. In pasteurized milk, the amounts were 53.0, 39.5 and 7.5 percent respectively.

Plaksanguansri (1991) studied microbial count of pasteurized milk stored for various times. He stated that prolonging shelf life of pasteurized milk is of great benefit to the Thai dairy industry. The storage life of processed and packaged milk from good-quality raw milk was found to be >21 days. The storage life reduced to 4

days due to contamination after pasteurization by a rapid increase in the number of microorganisms. In slightly contaminated pasteurized milk, the number of microorganisms gradually increased with increasing storage time. The numbers of psychrotrophs, mesophils, thermodurics and thermophileis that caused spoilage of pasteurized milk were 2.8108-8.6' 108, 2.9×108-9.9×108, 1.6× 103-1.4×105 and 49.70× 102 cfu. per ml respectively.

Ademollo *et al.* (1992) surveyed on the thermoduric microflora in milks subjected to different heat treatments. The scientist examined the thermoduric microflora in 60 pasteurized milk samples: 52 of these samples were pasteurized milk temperature. (71^0C/ 15s, 74^0C/15s, 77^0C/15s, 80^0C/15s) and 8 samples were collected after industrial pasteurization at 77^0C/ 15s. the result showed that the presence of thermoduric bacteria is closely bound up with the microbial contamination of the milk before the pasteurization.

Siva *et al.* (1993) conducted an examination to monitor the microbiological status of pasteurized milk. They found that the plate count of 10 samples of pasteurized milk varied from 10.000 to 62,000 with an average of 3.7±0.5 4×10,000 cfu/ml and the coliform count in 10 samples varied <10 to 80 with an average of 22±8.13 c.fu/ml. They further confirmed that the coliform counts were significantly correlated with the total plate count in pasteurized milk (r=0.754.).

Sasano *et al.* (1993) conducted an experiment on the standard plate counts (SPC) and counts of (I) psycrotrophic, (II) mesophilic and (III) thermoduric bacteria in raw milk produced, from 1990 to 1992 in Hokkaido prefecture, Japan, in Jan. and Aug. (n=1063). Average counts for SPC and (I) - (III) respectively were 26.9×10^3, 7.1×10^3, 18.7×10^3 and 1.9×10^3/ml. SPC comprised approximately 25, 68 and 7% (I), (II) and (III) respectively. The percentage of samples equalling or exceeding particular count levels were as follows. SPC, 72.0% for $\leq 3\times10^4$ and 97.5% for $\leq 110^5$/ml: (I), 67.9 for $\leq 5\times10^3$ and 85.0 % for $\leq 1\times10^4$/ml ; (II), 83.3% for $\leq 3\times10^4$ and 98.6% for $\leq 5\times10^5$/ml and (III), 67.3% for $\leq 1\times10^3$ and 91.5% for $\leq 5\times10^3$ per

ml. Relationships between SPC and (I)-(III) were r=0.469, 0.862 and 0.348 respectively.

Patel (1993) tested 21 samples each of foremilk and middle milk from buffaloes, 15 fresh dung samples and 10 rinse samples each from milk cans that had been, left unwashed or Washed at the collection point, for total plate (TPC) and coliform (CC) counts. Mean TPC and CC resp. were 2.3 x 10^5 and 5100 c.f.u./ml in foremilk 2.3×10^5 and 1900 cfu./ml in middle milk, 4.1×10^{11} and 1.9×10^{10}) cfu./ml in dung, 1.7×10^7 and 0. 11×10^6 c.f.u. /ml in rinse from unwashed milk cans and 1.1×10^6 and 1.6×10^4 cfu./ml in rinses from washed cans. CC was correlated with TPC in foremilk (r= -0.446, P<0.05) and in rinses from unwashed cans (i= 0.663, p<0.05). It is recommended that the 1st 5 streams of milk secretion should be excluded, that contamination of milk with dung should be avoided by pre milking udder and teat washing and that cans should be washed with potable water before being used for milk collection.

Diman (1993) demonstrated on the effect of refrigeration conditions on the level of bacterial contamination of milk. The effects on levels of bacterial contamination of milk of different refrigeration temperature (4, 10 and 15^{0}C) during primary processing on farms were investigated. Refrigerating milk to 4^{0}C caused the greatest decrease in bacterial numbers, although it was considered that refrigeration to 10^{0}C was adequate. Decreased bacterial contamination was associated with a decrease in the titratable acidity of milk. Refrigeration was found to have little effects on numbers of coliform bacteria in milk.

Abd-El-Ghani (1993) examined on the bacteriological quality of raw market milk in rural areas of Giza Province. 60 raw milk samples obtained weekly between Oct. 1989 and June 1990 from vendors about 50 km NW of Giza City (Egypt) and mean standard plate count (SPC) of 14 x 10^7, coliform count 65 x10^4C, staphylococci count 4 x10^3 and thermoduric count 85 x10^6 cfu/ml; coliforms were found in 85% and staphylococci in 65% of samples but no salmonellae were found. Of 200

farms, both before and after washing and, disinfection. Mean SPC and CC respectively in the raw milk were 1.7×10^4 and washing with water and 4600 and 15 after washing with disinfectant. Bacterial counts of bedding, udder, milking machine and hands eliminated by washing with disinfectant, and these decreased counts were reflected in lower counts in the raw milk.

Saitanu *et al.* (1996) collected 266 samples of raw milk (i) directly from dairy farms and (ii) at the corresponding milk collection centres in Thailand and examined microbiologically. Total bacterial count and coliform count were lower for (i) than for (ii). For (i) and (ii) respectively, 77.85 and 61.97% of samples had a total bacterial count of <50000 cfu. /ml, 69.44 and 57.33% had a coliform count of <1000 cfu /ml. 85.63 and 85.24% had psychrotrophic bacterial counts of <3000 cfu/ml and 98.31 and 90.250,/o of samples had thermophilic bacterial counts of <300 c.f.u./ml.

In another experiment, Slaghuis (1996) studied the contamination of milk of the three main sources (udder interior, and teat exterior and milking storage equipment) and they were concluded from a literature survey. The conclusions were as follows: most pathogens in the raw milk originate from the interior and exterior of the udder, with the udder interior being the main source of mastitis pathogens; contamination from the exterior of the udder originates from faces, feed, bedding, grass and soil; udder preparation can reduce teat contamination by up to 90%; cows at pasture have a lower level of teat contamination than have housed cows; high numbers of bacteria originate from milking and storage equipment, and storage at low temperatures favours growth of psychrotrophic micro-organisms; and raw milk quality, in general, is highly dependent upon hygienic production conditions.

Pandey *et al.* (1996) carried out an experiment on the sanitary quality and somatic cell count of raw milk which collected from 95 dairy farms applying milk to the Dairy produce Baord in Lusaka, Zambia. The standard plate count of raw milk ranged from log 7.66 to log 9.15 per ml of milk log 6.-06 to 7.20 per ml of milk and

the staphylococcal count ranged from Log 5 to 6.38 per ml of milk. They reported that the sanitary quality of raw milk was not acceptable in approximately 45% of dairy farms around Lusaka.

Mutukumira *et al.* (1996) carried out an experiment on chemical and microbiological analysis of raw bulk milk from 34 producers collected from the Nharira/Lancashire milk collection centre, and found that the coliforms bacteria were 3.2×10^2 to 2.3×10^5 and lactic acid bacteria were $< 1\times10^3$ to 2.9×10^6 cfu/ml.

Lakhani and Jogi (1996) conducted an experiment in which they studied the bacteriological quality of raw milk in Murrah buffalo that obtained by machine milking vs. hand milking. They resulted that standard plate count (SPC) and coliform count were significantly higher in hand milking than machine milking in the initial stage (4.522± 0.174 vs.3.43 ±0.15 and 3.687 ±0.2 vs. 2.32 ± 0.133 log/ ml respectively).

Azizur (1996) studied on the total count and coliform count of bacteria in per ml pasteurized milk processed in different dairy plant of Milk Vita and found that the average total viable count were 1821.42, 4150.00 & 15442.85 cfu. /ml and the average coliform count were 0.5, 3.42 & 146 cfu. /ml of Dhaka Dairy Plant (DDP), l3aghabarighat Dairy Plant (BDP) and Faridpur Pasteurization Plant (FPP).

Saharia *et al.* (1997) conducted an experiment on the bacteriological quality of milk assessing by coliform counts made on 263 raw milk samples collected from units at various altitudes and seasons, and with good or wooden flooring. The overall average count was 378.7±13.9/ml. Location had no significant effect, but significantly higher counts were associated with the monsoon and post monsoon periods, and houses with wooden floors.

Garg and Mandokhot (1997) analysed 86 samples of raw milk (67 from, local vendors, 6 from vendors at organized dairy units and 13 from a local milk plant) for standard plate count (SPC), methylene blue reduction time (MBRT) and titratable acidity (TA), 41 samples that were found to be adulterated with

carbonates/bicarbonates all had TA within the legal limit of 0.17 percent. This showed that TA was unreliable as a platform quality control test at Milk collection centres. In comparative grading of the samples on the basis of MBRT and SPC, there was agreement between the 2 methods for 30 samples only; the MBRT placed 54 samples in a higher and 2 in a lower category than did the SPC. The W graded 64 samples as poor (SPC>5 million per mi.) compared with only 17 per cent when the MBRT was used. Thermoduric counts determined for 26 samples ranged from 14-3 million per ml and were <200 pet ml in only 6 samples.

Cempirkova (2003) studied the microbiological quality of raw cow's milk in relation to the utilized technology of breeding .This study was conducted to assess the impact of the utilized breeding technology and method of milking on the milk quality. In 2002, bulk milk samples from 4 farms were monitored. On 2 farms, the cows were housed in loose boxes and milked in parlours. In the other 2 farms, the cows were housed in stanchion barns and milked with pipeline milking machines. The microbiological indicators of milk quality were lower for both technologies. The average annual total bacterial count (TBC) was <15x103 c.f.u./ml, whereas the psychotropic bacterial count (PBC) was <2.2x103 c.f.u./ml. The coliform count was <70 c.f.u/ml.A more significant seasonal variation in microbiological indicators with a tendency to deteriorate in winter months was apparent in the milk of cows housed in stanchions barns and milked with pipeline milking machines. The average values of the relative index pi (PBC/TBC) ranged from 0.21-0.26. The average somatic cell count (SCC) annual values were lower (217x103 to 232x103 SC/ml) for cows housed in loose boxes and milked in milking parlours than for cows housed in stanchion barns and milked with pipeline machines (285x103 to 295x103 SC/ml). Pasturing, which took place in one of the farms with stanchion barns from May up to the beginning of November, significantly improved all the monitored milk quality indicators. Housing in loose boxes and milking in parlours provided better conditions for the production of good quality milk.

Reithmeier *et al.* (2004) studied the bacterial load of several lying area surfaces in cubicle housing systems on dairy farms and its influence on milk quality. For various reasons (lower amounts of straw, cow comfort, easy handling) new bedding systems such as geotextile mattresses are becoming more common in today's dairy farming. By analysis of bacterial counts of 5 groups of bacteria (aerobic mesophilic count, anaerobic spore formers, enterococci, Staphylococcus aureus and enterobacteriaceae) we evaluated the influence on the microbiological quality of milk for 5 different types of lying areas. The study was performed on 25 dairy farms by comparing bacterial counts in samples of the lying area surface, teat surface and milk from cows lying on the respective surfaces in a summer and a winter trial. Significant correlations between the bacterial load of the lying area surface and the teat surface were found for some of the bacterial groups, but only for anaerobic spore formers a significant correlation between lying area surface, teat surface and individual bucket milk could be shown. Seasonal variations of the bacterial load were observed in milk samples. Increased counts of anaerobic spore formers were obtained in winter, the opposite was true for enterococci and enterobacteriaceae. In general, we could not find any significant influence on the microbiological milk quality by the different bedding systems. Straw mattresses did not differ significantly from geotextile mattresses.

2.4 Shelf life and keeping quality

Mourgues *et al.* (1983) studied the samples of raw milk from three farms and three dairies in the region of Paris. Samples were pasteurized at 63 deg. C for 30 minutes and stored at 6 or 8 deg. C. The keeping quality of the 52 pasteurized milk samples were assessed from a number of days at 5 or 8 deg. C until the total bacterial counts increased to more that 30.000 per ml and a flavour defect developed. From the result it was concluded that when the raw milk contains 10,000 heat resistant bacteria per ml was pasteurized. it could only be stored at 8 deg. C for 13 days without development of flavour defects. However, if the raw milk containing 1,00,000 heat-resistant bacteria per in] was pasteurized, it could only be stored at 8

deg. C for 10 days. Lowering the storage temperature to 6 deg. C the shelf-life of the milk could be increased to 23 and 18 days, respectively.

Schroder (1983) suggested on osrigins and levels of post pasteurization of milk in the dairy and their effects on keeping quality. Bacterial post pasteurization contamination with psychrostrophic Gram-negative rods (GNR) was measured in commercial milks before and after transfer to retail containers. The tanks of pasteurized milk feeding the filling nits contaminated milk less often (39% of samples) but usually at a higher level than the filling units (92 % of samples). The number go GNR present had a considerable influence on the shelf life of milk, and the range found in commercially pasteurized milk was reflected in a wide range of shelf lives.

Hur (1984) carried out an experiment on keeping quality of raw milk during the period from July, 1983 to July 1984. He transported milk from 9 dairy farm by ice box and stored in underground water cooled tank or in a cooler for individual can or in a bulk tank cooler. The temperature of milk cooled by the different methods tended to decrease as the trail continued and was 23-10, 20-7, and 18-6^{0}C for the 3 methods respectively. Titratable acidity varied between 0.20 and 0.17% with the underground cooled and between 0.18 and 0.14% with the unit cooler and the bulk tank. The preparation of samples with low titratable acidity was greatest with the bulk tank. All the samples were (-) ve in the alcohol test but 17 gave doubtful results, 10 stored in the underground cooler and 7 stored in the unit cooler. He concluded that the best results were obtained with the bulk tank.

Spillmann (1985) studied the effects of raw milk quality, heating conditions, packaging materials, temperature during storage and distribution on the keeping quality of pasteurized milk and cream. Model calculations concerned with recontamination by psychrotrophs during storage at 4 and 8^{0}C are presented, and suggestions for improvements in the keeping quality of pasteurized milk are made. Methods for detecting post-pasteurization recontamination are also discussed.

Kodikara (1987) studied the effect of storage temperature of milk on bacterial count and keeping quality of milk, and the maximum length of time that will could be kept at room temp. (27^{0}C) without deterioration of quality in Sri Lanka. The total viable count (TVC), coliform, faecal coliform and psychrotrophic counts, resazurine test score, and percentage acidity were measured on 2 sets of milk samples stored at room temperature, (27^{0}C) and 4^{0}C. The results show the rate at which the above bacterial types increase at the 2 temp. studied. Both the resazurine test score and the percentage acidity showed that milk having a TVC of 263 x1012/ml, which is even less than the average TVC of raw milk for the kandy area, Sri Lanka, could be kept for only 2 h at 27^{0}C without deterioration of quality.

Huh (1988) carried out an experiment for assessment of methods to identify post-pasteurization contamination and to predict potential shelf-life of fluid milk products. This study focused on the identification of psychrotrophic post-pasteurization contamination and the prediction of potential shelf- life of fluid milk products. The low fat (1 % and 2 %) milks had more potential shelf-life problems than whole milk and skim milk. Preliminary incubation is an excellent tool for predicting keeping quality for fluid milk products.

Everhard and Gallman (1988) conducted the laboratory and field experiment by which they confirmed that recontamination reduced keeping quality of pasteurized milk. It was not possible to entirely exclude recontamination in practical conditions; milk pasteurized at high temperature has a shorter shelf-life after recontamination than low temperature- treated milk; permanent refrigeration at <4^{0}C inhibited growth of recontaminated bacteria.

Koshy and Padmanaban (1988) carried out an experiment in which 200 samples of market milk and 200 samples of pasteurized standardized milk from a cooperative were stored at 5^{0}C or at room temperature for up to 96 h. The commercial samples were first pasteurized at 63.5±0.5^{0}C for 30 min. After 24, 48, 72, and 96 h storage at 5^{0}C, 84, 44, 11 and 0 respectively of the samples from the cooperative were

negative for the test, compared with 58, 15, 0 and 0 of market milk samples. After 6, 12 and 18 h storage at room temperature, 79, 29 and 0 respectively of the cooperative samples were negative for the test, compared with 63, 10 and 0 of market milk samples.

Sharma and Lavania (1988) studied on keeping quality of milk at room temperature. Keeping quality of 30 samples from milk vendors, small dairies and individual milk producers was studied by them. Each sample was divided in to 3 parts: one was pasteurized (71^{0}C for 16s), one boiled and one left untreated. Each was incubated at room temperature, and analyzed after 4, 6, 8, 10 and 12 h. Raw milk from small dairies remained acceptable as just by the clot-on-boiling test, for up to 8 h. boiled and pasteurized milks could be stored for 10-12 h. (December to May). Vendors' milk was of the poorest quality, with that obtained from individual milk producers being of intermediate quality.

A study was undertaken by Koshy and Padmanaban (1990) to compare the keeping quality (KQ) of pasteurized milk samples at 5^{0}C and at room temperature with the changes in p^H and acidity, as well as the thermoduric (TD) and thermophilic (TP) bacteria load. The results indicated a positive correlation between TD and TP counts and KQ, and also a positive correlation between TD and TP counts, and the time taken to attain the critical p^H and acidity when milk was found positive to clot-on-boiling test. This indicated a direct relationship between TD and TP counts, and KQ of milk samples. TD and TP counts could therefore be used as an indicator of KQ in pasteurized milk samples.

Bhardwaj *et al.* (1991) showed that average keeping time of raw milk packaged in food-grade low-density polyethylene packets, cooled to 5±1^{0}C by immersing the packages in ice-cold water and stored in insulated boxes was 45, 60, and 33.22 h respectively. Such a long keeping time indicated the feasibility of milk collection from consumers through retail shops.

Buenaventura *et al.* (1991) obtained winter and summer liquid milk samples (whole, low fat and skim) from 19 plants representing 5 geographical areas of the USA. The samples were evaluated by a sensory panel for up to 14 days of storage at 6.5^{0}C to determine if the quality was still acceptable. Using this approach, a greater percentage of summer milk samples (94 to 158) spoiled within 14 days than did winter milk samples (64 of 156). When samples were evaluated for acceptability, based upon the code date utilized by the processor, no difference was observed between spoilage rate of summer (52 of 158) vs. winter milks (53 of 156). Minnesota milks maintained their quality the longest while samples from Kansas and the NE had the shortest shelf-lives. Characterization of flavours in the milks revealed that summer milks tended to go rancid during storage while winter milks became bitter.

Sims *et al.* (1991) conducted an experiment on the impedimetric analysis of quality and shelf-life of milk pasteurized by a continuous microwave treatment, beating commercial raw milk to 72, 80 or 85^{0}C for 0, 15 or 30 s by a continuous microwave ~process in 2450 - MHz, 700-W oven resulted in initial reductions in microbial counts, followed by increases in standard plate count and psyclitotropilic bacterial count (PBC) during storage at 4^{0}C; at day 21, 93 per cent of samples has PBC of 105-106 per ml. IDT decreased with storage, based on the IDT of milk pre incubated of 18'C for 24 hours. The potential shelf life of samples was 7 to 14 days.

Ashenafi and Beyene (1993) studied about milk, collected aseptically from all quarters of cows and distributed in to a bottle cleaned with tap water, dried and smoked, a bottle cleaned with warm water and a bottle cleaned with water and detergent. Milk kept in a sterilized bottle served as a control. Initial counts of aerobic mesophilic bacteria ranged between 103 and 104 c.f.u./ml and increased steadily, at varying rates , in collected in all containers. Aerobic mesophilic bacteria and coliforms reached a level of 108 c.f.u./ml after approximately 36h in milk held in smoked containers, whereas they reached the level in <24 h in milk collected in the other containers. Washing udders with warm water (52^{0}C) or with 3.58 savlon

solution did not improve the keeping quality of raw milk. Cleaning of containers with water alone or with detergent did not markedly affect the rate of proliferation of initial flora, but smoking had a significant inhibitory effect particularly in the first 24 h. smoking of milk containers may thus improve the keeping quality of raw milk.

Borde-lekona *et al.* (1994) determined the keeping quality of pasteurized and high pasteurized milk regarding PPC. The aim was to heat-treat milk at 72^0C for 15 s and at 115^0C for 2 s, in the virtual absence of post-processing contamination (PPC), and to determine its keeping quality at 2 different storage temperatures (2 and 10^0C). It was found that there is scope for improving keeping quality by reducing PPC.

Barnard *et al.* (1995) reported that a list of processing procedures and hygienic practices to help milk processing plants to manufacture pasteurized milk that has acceptable flavour and keeping quality after storage at 7.2^0C for 21 days. These procedures serve as a guide to identifying hazard analysis critical control points or the good manufacturing practices for the plant.

Lakhani and Singh (1998) stated that bacteriological quality of raw milk obtained by machine was significantly better than that of milk obtained by hand. The keeping quality of raw milk obtained by machine and by hand respectively averaged 10.70 and 9.27 h on the basis of various bacteriological tests and 10.6 and 9.3 h on the basis of COB test.

Solanky *et al.* (2003) conducted an experiment to determine the effect of lactoperoxidase (LP) system at two different levels of SCN and H_2O_2 (25 :15 and 70 :30) on the population of mesophilic and thermophilic spores, total and proteolytic psychrotrophic spores in raw milk. A strong bacteriostatic effect of LP system was observed on high heat and low heat resistant mesophilic as well as thermophilic spores. The sstem was further found to be bactericidal for psychrotrophs. Considering the very high bacterial population of raw milk available

in different dairy plants in India, LP system is a unique opportunity to preserve innate microbilogical characteristics of raw milk intended for UHT processing.

Islam *et al.* (2003) an experiment to investigate the effect of refrigeration on the keeping quality of raw and pasteurized milk. Collected milk samples were pasteurized at 63 degrees c for 30 min. Raw and pasteurized milk samples were placed in a refrigerator maintained at 7 degrees c. The total viable bacteria of raw and pasteurized milk were 5.915 and 4.094 log c .f.u./ml, which increased to 6.648 and 4.579 lig c.f.u./ml, respectively. The amounts of coliform bacteria in raw and pasteurized milk were 2.043 and 2.546 log c.f.u./ml,respectively, but after 10 days the numbers increased to 3.070 and 2.607 log c.f.u./ml, respectively. After 10 days, pasteurized milk displayed only a marginal increase in shelf life compared to raw milk.

2.5 Milk treatment and preservation

Large proportion of milk produced in the world is preserved by various process such as physical means (pasteurization, homogenization, cooling and irradiation etc.) and chemical means (H_2O_2, lactoperoxidase, CO_2, $K_2Cr_2O_7$, and $NaHCO_3$).

2.6.1 Milk preservation by physical means

Eberhard and Gallman (1988) carried out an experiment where pasteurized milk from 2 large dairies in Switzerland was kept for 12 days at 8^0C, there was no direct relationship between bacterial count and organoleptic grading, as serious organoleptic defects were not observed until bacterial count exceeded 107/ml. Although the milk from one of the dairies contained large numbers of spores and reached bacterial counts over 106/ml in 5 days, it had a consistently higher orgnoleptic score then the other dairies milk until day 7, after which it rapidly deteriorated.

Cuoghi (1993) modified the hydrostatic pressure treatment as an alternative food preservation process to heat treatment. It has the advantage of improved retention of taste, colour and texture compared with heat treatment. It also affects food

constituents; proteins, lipids, and starches may undergo conformational changes that could result in the development of new products. Recent research involving milk and milk products has been concerned with high-pressure treatment to reduce microbiological contamination without affecting organoleptic and nutritional characteristics. This process could be utilized in the manufacture of hard cheeses from 'raw milk' e.g. Grana, Emmental, and Gruyere. Changes in coagulation properties and gel firmness occur in pressure-treated milk. Therefore further studies need to be carried out to assess the full effects of high-pressure treatment on the cheese making process.

Guulsimonsen *et al.* (1996) examined the instant cooling/continuous process compared with normal cooling/batch process over a 2-5 h period cooling to 4^0C, resulted in slightly slower bacterial growth and slightly slower hydrolysis and oxidation of milk fat. Instant raw milk cooled and stored at 2.5^0C maintained acceptable quality for approximately 1-2 days more than when normal cooling over a 1.5 h period and storage at 4^0C was used.

2.6.2 Milk preservation by chemical means

Kang *et al.* (1983) stated that addition of H_2O_2 (at 0.02%) to fresh milk on the farm, protected the milk from microbial spoilage during 12 h at 20-30^0C. Added H_2O_2 was completely decomposed in about 5 h without addition of catalase, indicating that this amount of H_2O_2 can be safely used as a preservative for farm milk during transportation to processing plants. Misra and Verma (1986) carried out an experiment on the shelf-life of raw buffalo milk treated with various concentrations of H_2O_2 (w/v) and stored at different temperatures.Among the four concentrations (0.05, 0.10, 020, and 0.03% w/v) of H_2O_2 used for preservation of milk, 0.10% was found to be sufficient for the purpose. At this concentration, buffalo milk could be stored up to 62, 32.5. and 16 h at 15deg, 2 deg, and 35 deg C respectively without any appreciable adverse effect on overall acceptable sensory quality.

Decomposition of H_2O_2 was found to have direct relationship with the temperature of storage.

Wang *et al.* (1987) conducred an experiment about the use of antibacterial lactoperoxidase (LP) system for preservation of milk quality. It was concluded that the separate addition of minute quantities of thiocyanate (SCN-) and hydrogen peroxide (H_2O_2) (approx. 12 and 8.5 ppm resp.) was quite successful in securing optimum activity of the LP system in bovine milk and might be utilized as a reliable means of increasing the keeping quality of raw milk stored at room temperature or refrigerated.

Bjorck (1987) discusses the use of H_2O_2 and the lactoperoxidase system to prevent bacterial spoilage of raw milk in situations where refrigeration is not available.

Rai and Jandal (1988) studied the effect of mercuric chloride on keeping quality of milk, covering effect on milk composition (protein, fat, lactose, and enzymes), milk colour, titratable acidity, specific gravity.

Jha and Verma (1988) studied the effect of addition of potassium sorbate (0.3%) to khoa, with or without nitrogen flushing, on flavour, microbial and chemical changes during storage at 30 ± 2^0C. They reported that addition of potassium sorbate increased the shelf life of khoa to 40 days. The microbiological and chemical changes during storage greatly influenced the flavour scores of khoa.

Narasimhan *et al.* (1989) examined the sweet cream butter which was treated as follows-(i) without preservatives (control), (ii) with 1000 ppm. potassium sorbate, (iii), (iv) and (v) with 2% NaCl+ 500, 750 or 1000 ppm. potassium sorbate respectively. They found that average yeasts and moulds counts (c.f.u. /ml) were (i) 11.14 x102, (ii) 3.77 x102, (iii) 8.00 x102, (iv) 3.67 x102 and (v) 1.32 x102. Average free fatty acid content (FFA) (%) was (i) 1.96, (ii) 1.19, (iii) 1.36, (iv) 1.19 and (v) 1.02. Sample (v) had the best keeping quality; yeast and mould count, and FFA were significantly lower than in (ii) and (iv) ($p<0.05$).

Huh (1989) conducted an experiment where milk samples were collected from retail outlets to study their microbiological and organoleptic properties. Av. Bacterial counts of the milk samples were: coliform count 2.42 cfu/ml; standard plate count 3500 cfu/ml; psychrotrophic bacteria count 2800 cfu/ml.

Hossain *et al.* (1990) carried out an experiment to determine a suitable level of hydrogen peroxide by which milk can be preserved for short periods of time in rural areas of Bangladesh. They reported that the acidity of the untreated milk was significantly higher ($p<0.05$) than that of the hydrogen peroxide treated milk sample. On the basis of clot-on-boiling and methylene-blue reduction tests the quality of hydrogen peroxide-treated milk samples was better than the untreated milk sample. Organoleptic evaluation also showed that taste, flovour and colour of hydrogen per oxide-treated milk samples were superior to that of the untreated milk samples after 24 h. They also reported that a concentrated range of 0.02 to 0.04% of hydrogen peroxide was the most effective level for milk preservation.

Lacroix and Lachance (1990) studied the effect of various humectants and Water Activity (AW) on proteolysis, yeasts and mold growth and shelf-life during cold storage of yoghurt. For this purpose, 27 experimental samples were prepared by adding hymectants to plain yoghurt according to a complete 33 factorial design comprising: NaCl (0, 4, 8%), sucrose (0, 7.5, 15%) and sorbitol (0, 7.5, 15%). They suggested that there were a significant relationships between AW, hymectant content and shelf-life of yoghurt during cold storage ($R2>0.90$). A 1% increase in salt, sucrose and sorbitol content of yoghurt resulted in about 7.7, 1.5, and 1.3 days increase in shelf-life resp. Similarly, a 0.01 decrease in AW increased shelf-life by 11.5 days. The impact of these results on optimization of the shelf-life of commercial yoghurt and on the development of more stable yoghurt products for industrial use is outlined.

Ambadkar and Lembhe (1991) reported that the addition of hydrogen peroxide to raw milk significantly increased its shelf-life without affecting its quality. 300 ppm.

H_2O_2 preserved the milk for 18 h. further increase in H_2O_2 concentration provided a greater safety margin. They also observed a direct relationship between the decomposition of H_2O_2 and the preservation period. The cost of preservation with 300 ppm. H_2O_2 was approximately 10 paise/ litre.

Kukde, *et al.* (1991) examined the effect of sodium bicarbonate on storage period and quality of milk. Sodium bicarbonate decreased the acidity of milk by its neutralizing effect rather than by its antibacterial effect. Addition of 0.8% sodium bicarbonate increased the storage period of milk to 30 h, if stored in an earthen pot at 21^{0}C, whereas at refrigeration temperatures 0.2% sodium bicarbonate increased the storage period to 45 h. storage of milk in an earthen pot with addition of 0.8% sodium bicarbonate is recommended.

Mahboob (1992) conducted an experiment on the keeping quality of raw milk with sodium bicarbonate as a preservative. He used three levels of sodium bicarbonate. 0.05, 0.10 and 0.15% along with one control sample. Room temperature in this trial ranged from 10 to 19^{0}C. from this experiment, it was found that acidity of control milk samples increased significantly than the milk samples preserved with sodium bicarbonate. Control sample was found good up to 12 h. where as milk on other treatments with preservative were good during the whole 24 hours of study. He concluded that sodium bicarbonate can be used to preserve milk at 0.05% level at least for 24 hours at room temperature.

Ambadkar and lembhe (1994) stated that the addition of hydrogen peroxide to raw milk significantly increased its shelf-life without affecting its quality. 300 ppm H_2O_2 concentration provided a greater safety margin. A direct relationship was observed between the decomposition of H_2O_2 and the preservation period. The cost of preservation with 300 ppm H_2O_2 was approximately 10 paise/litre.

Kumar and Mathur (1994) studied about the proteolytic changes in raw buffalo milk preserved by LP-system. When pooled buffalo milk was preserved by activation of the lactoperoxidase no statistically with 25:15 or 70:30 SCN-: H_2O_2 and stored at

30^0C for up to 16 h. were observed; no statistically significant changes were noted in proteolysis (interms of tyrosine value) or in levels to total N, non casein N, protease- peptone N or non-protein N.

Sarkar and Misra (1994) reported that the preservative effect of the LP system decreased with increase in storage, but it is considered a suitable method for raw milk preservation under field conditions in tropical counties. It is recommended that 20:10 and 20:20 ppm. SCN-:H_2O_2 be used to activate the LP system for preservation of cow and buffalo raw milk respectively.

Abd-El-Hady (1995) observed that the effects of heating and storage of milk on the decomposition of H_2O_2 was dependent upon the initial concentration of the H_2O_2, the time and temperature of heat treatment and the storage temperature. During storage at 32^0C of milk containing 0, 100, 150, 200, and 250 ppm H_2O_2 respectively, cow milk coagulated after 10, 14, 16, 18 and 20 h and buffalo milk after 8, 12, 14, 16, and 18 h, by which time total bacterial counts had increased to >109 c.f.u./ml.

Jandal (1996) studied on lactoperoxidase /onion extract/ethanol system in the preservation of raw buffalo milk. In this system, buffalo milk samples (100 ml) were prepared with 12 mixtures of onion extract (1-12 ml) and ethanol (0.5-3 ml). The effect of treatment was evaluated by examining titratable acidity (TA) and clot-on-boiling tests 3-hourly for 21 h. The storage life of buffalo milk was increased to>15 h by LOE-treatment compared with ≥ 3 for raw buffalo milk. Buffalo milk samples treated with LOE had not coagulated after 15 h of storage. In untreated buffalo milk samples, there was a progressive development of TA with increase in storage time. In buffalo milk samples treated with LOE the development of TA was delayed and samples remained acceptable for up to 15 h. Results indicated that buffalo milk produced in rural conditions and treated within 2 h of production with LOE could be stored for 15 h. It was concluded that the LOE system can be used to preserve milk produced in rural areas under conditions where cooling facilities or

chemical preservatives are not available, or transport distances are long, or where an extended storage time is required for cooled milk.

Jandal (1997) observed new methods for preserving milk. New methods such as the lactoperoxidase/onion extract/ethanol (LOE) system and lactoperoxidase/garlic extract/ethanol (LGE) system were described as rate of their bactericidal mecnanisms. Experiments including titratable acidity, clot-on-boiling test and alcohol coagulation were performed on milk treated with the 2 new systems. It is concluded that the LOE and LGE systems are suitable alternatives for raw milk preservation in tropical countries, since no cooling or addition of chemical preservatives is required, and there are no potential health risks.

Biswas *et al.* (1997) carried out an experiment about the effect of banana leaf on the keeping quality of raw milk. For this purpose, milk samples were collected from market and BAU, Dairy Farm and milk samples were stored up to spoilage time; and physical, chemical and microbiological study and agitation effect was monitored for observing by effect of banana leaf treated milk was good up to 12 to 14 hours. And suggested that banana leaf might be used randomly as a short-time-cost-less milk preservatives under village condition.

Pal and Ghatak (1998) observed that the effect of the lactoperoxidase system (LP) activated at 15:10, 20:10, 25:15, 45:20 ppm (SCN: H2O2) for the preservation of cow and buffalo milk at 30^0C with the help of titratable acidity, alcohol and clot-on-boiling test. Activation of LP at all levels increased the keeping quality of cow and buffalo milk. Cow and buffalo milk activated at 45:20 ppm (SCN: H2O2) gave the maximum increase in keeping quality of 8h.

Saha *et al.* (1998) studied on the preservation of raw milk with hydrogen peroxide (H_2O_2) (and sodium bicarbonate ($NaHCO_3$) for rural dairy farmers. For this purpose, they were concluded two experiments. In the first experiment, they preserved milk samples with 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06. per cent (H_2O_2). They concluded that 0.04 to 0.05 percent (H_2O_2) was enough to preserve milk

samples up to 24 hours. In the second experiment, they preserved milk samples with 0.025, 0.05, 0.075, 0.10, 0.125 and 0.15 percent $NaHCO_3$ and they concluded that 0.1 per cent $NaHCO_3$ was suitable level for preserving milk up to 24 hours.

Barrett *et al.* (1999) studied the kinetics and mechanism of action of the lactoperoxidase system (LPS) in pasteurized milk. They reported that the keeping quality of milk pasteurized at 72°C for 15 s was better than that of milk heated at 80°C for 15 s. They also detected that higher levels of hypothiocyanite (the major antimicrobial agent produced by the lactoperoxidase system) in milk processed at 72 than at 80°C, which supports the theory that the LPS has a role in the keeping quality of pasteurized milk.

Pongracz (2000) reported that cooling is the best and required method of raw milk preservation until pasteurization, but in areas where it is not possible be apply cooling facilities there is a need for a method to maintain the quality of raw milk. Milk contains several antibacterial components such as immunoglobulins or other non-specific components like lysozyme, lactoferrin and lactoperoxidase. Lactoperoxidase provides a natural antibacterial effect on fresh milk, but its activation is mediated by both thiocyanate and hydrogen peroxide.

Farid (2003) concluded that sodium chloride could be used as a preservative of milk for short term milk preservation. A level of 1.0% sodium chloride addition had no bad taste and improved the shelf life 3 hours more than that of the control samples. At the same time addition of salt up to 4.0% could preserve milk 11 hours more than that of the untreated milk samples. But this high level had high salty taste in milk and are not recommended for direct consumption but recommended for manufacture of dairy products like butter, cheese etc.

Radha *et al.* (2003) observed the preservation of milk samples with formalin -- effect on milk constituents. This study was undertaken to assess the effect of formalin on the percentage of fat, total solids, and solids not fat in cow and buffalo milk. Formalin was added at a concentration of 0.4%. Formalin treated cow and

buffalo milk samples were preserved up to 90 days without curdling. No colour change was observed in the milk samples. The pH values of control samples were 6.58ñ0.02 and 6.76ñ0.01 for cow and buffalo milk, respectively. The values decreased significantly ($P < 0.01$) from day 0 to day 90 of storage. On addition of formalin, the titratable acidity of both cow and buffalo milk increased, and on subsequent storage, the acidity continued to increase slowly. At 90 days, the values became 0.27ñ0 and 0.25ñ0.01, with the change being significant ($P < 0.01$). Both cow and buffalo milk samples remained clot on boiling test (COB) negative for the entire storage period of 3 months at room temperature. On addition of formalin, the fat percentage decreased to 3.32ñ0.1 and 6.13ñ0.22 in cow and buffalo milk samples, respectively. At 90 days of storage, the values became 3.38ñ0.09 and 6.18ñ0.22 in cow and buffalo milk, respectively. This change observed through the Gerber method was insignificant. Using the Milko-Tester, a slight decrease in fat percentage was observed on addition of formalin (3.33ñ0.10 and 6.13ñ0.21). Up to 30 days of storage, there was no significant change in fat percentage. On addition of formalin, the total solids in cow and buffalo milk samples were 12.46ñ0.13 and 15.58ñ0.49, respectively. At 90 days of storage, the values were 12.51ñ0.12 and 15.59ñ0.49. The content of total solids did not show any significant change neither on addition of formalin nor during storage. On addition of formalin, the solids not fat percentages of cow and buffalo milk samples increased, with values of 9.14ñ0.12 and 9.45ñ0.29 on day 0, respectively. At 90 days of storage, the values were 9.12ñ0.10 and 9.41ñ0.28, respectively. However, the change was insignificant. On addition of formalin, lactometer reading decreased to 29.0ñ0.53 and 27.5ñ1.09 on day 0 in cow and buffalo milk, respectively. At 90 days of storage, the values became 28.67ñ0.42 and 27.50ñ1.09, respectively. Changes in lactometer reading were insignificant.

Radha *et al.*(2004) studied the effect of Bronopol as milk sample preservative-composition and physicochemical properties of cow and buffalo milk samples Bronopol (2-bromo-2-nitro-1, 3 propanediol) at 0.1% concentration was evaluated

as a milk sample preservative. Cow and buffalo milk samples preserved with bronopol could be stored for 24 and 16 days at room temperature, respectively. No significant change was noticed in the fat, total solids and solids not fat percentages of both cow and buffalo milk samples. An increasing trend was observed in titratable acidity from day 0 to the last day of storage. Furthermore, this study revealed the short-term preservative effect of bronopol and its suitability for both conventional and instrumental methods to estimate fat percentage.

From the above mentioned reviews, it is apparent that the physical, chemical and microbiological quality of milk supplied by farms and established dairies is better than those supplied by rural farms and village milk vendors. It is also found that the contents of milk vary individual to individual, breed to breed and region to region. Milk contents also vary due to feed, season, milking interval etc. Although milk is a highly nutritious food but its quality easily be deteriorated if we do not apply any technique to preserve them for increasing their shelf life. Hence, the present study was undertaken to compare and to evaluate the physical, chemical and bacteriological qualities of milk collected from selected areas of Bangladesh as well as shelf life of milk preservation under village condition.

CHAPTER 3
EXPERIMENT

Chapter 3

GENERAL MATERIALS AND METHODS

Statement of the problems

Milk is a highly nutritious food, as because it contains all the nutrients required for normal functioning of the body system. More over, there are some nutrients, like casein and lactose, which are not found in any kind of food except milk. Although milk is highly nutritious but we have huge shortage of milk. At the same time we don't have enough idea about the quality of milk we are consuming daily from local markets. On the other hand a huge amount of milk undergoes spoilage each year due to lack of preservation facilities. Farmers of rural areas used to some indigenous technology for milk preservation. But no scientific study has been done in the past to monitor the feasibility of using indigenous technology for milk preservation. So, considering everything, the present study was designed to judge the quality of raw milk found in different areas of Bangladesh and at the same time to develop milk preservation technology for rural farmers.

Site and location of experiment

In order to achieve the stated objectives of the project a series of research works have been carried out during the study period. Milk samples were collected from different local markets of Mymensingh district and northern part of Bangladesh (Sirajgong & Pabna district). The analysis of milk samples were carried out in the Dairy Technology laboratory of the Department of Dairy Science, Bangladesh Agricultural University, Mymensingh. Experiments regarding milk preservations were also conducted in the same laboratory of the same Department.

Name of experiments

A total of 6 experiments were conducted. Out of which 2 were on milk quality and 4 were connection of milk preservation technology.

Sample Collection

Milk samples were collected from different sources, in different time to time and were analyzed in the Dairy Technology and Dairy Microbiology Laboratory of the Department of Dairy Science, Bangladesh Agricultural University, Mymensingh. Milk samples were also analyzed in the Quality Control Laboratory of Bangladesh Milk Producers Co-operative Union Limited (Milk–Vita).

Design of experiment and data analysis

The design of experiments varied depending on the nature of the experiment. Milk is a homogenous material, in most of the time the design used was Completely Randomized Design (CRD) and Randomized Complete Block Design (RCBD) design was also used to conduct the experiment. Analysis of variance tests were done to see the statistical differenes within treatments. In case of significant difference LSD test were conducted.

Experiment 1 Qualitative characteristics of market milk collected from some selected areas of Mymensingh district.

INTRODUCTION

Milk is one of the nature's almost complete food. It contains all the ingredients nccessary for normal functioning of thc baby systcm for all agcs of pcoplc. Ncw borne bady can survive depending only on milk upto six months without having any othcr foods. Growing childrcn rcquirc it for thcir normal growth and dcvclopmcnt. It is also an unique food for adults and older people.

The main components of milk are water, fat, protein, lactose, minerals and vitamins. The water of milk is similar to the ordinary water and perform similar functions. Milk fat contains 40% saturated and 40% unsaturated fatty acids, but most of the animals fats like beef fat, mutton fat etc. consisted of mainly with saturated fatty acids and their melting point is usually higher than milk fat. For this reason quality of milk fat is better than other animals fats. Milk contains unique quality protein. Major of milk protein is casein about 80% of total protein) which is not found in any other foods. On the other hand remaining 20% is albumin and globulin fractions. Milk protein contains all the essential amino acids and for this reason quality of milk protein is better than any other protein. Another unique component of milk is lactose, which is a disaccharide and responsible for proper nourishment of brain tissues and nerves. Lactose is not found in any other foods. This is the speciality of milk. Milk also contains good amount of minerals. It is an abundant source of calcium and phosphorus but only limiting in iron. All fat and water soluble vitamins are also present in milk and performing various functions for the body system.

Although milk is a very highly nutritious food for all ages of people, but its availability is very low in our country. Hardly we can get about 40 ml of milk/head/day but according to GOB (1999) recommendation an adult people

should consume about 300 ml of milk/day. At the same time the quality of milk we are consuming from local market is unknown. Due to various reasons there are lot of possibilities for deteriorating the quality of milk. Villagers have very limited knowledge about hygienic milking, Vendors who are supplying milk to the city center also adultarates milk by adding water. Some times they mix chemical preservative with milk to increase the shelf life. Some sporadic research works have been done in our country to monitor the quality of market milk by Islam (1984), Alam (1989), Rahman (1995), Ali (1998) and Azad (1998).

But unfortunately no systematic research have been carried out to evaluate the quality of market milk. Scientists of other countries have done lot of works on their market milk Ghos (1965), Borges (1978), Hur (1984), Bjorck (1987), Kukde (1991) and Barnerd (1995) but their result will not be applicable in our country condition. So, in order to get idea about the quality of milk we are purchasing from local markets we have to carryout research works in this connection. Hence, the presence research was under taken to judge the quality of market milk available at different local markets of Mymensingh District.

MATERIALS AND METHODS

The present experiment was conducted at Dairy Technology and Microbiology Laboratory of the Department of Dairy Science, Bangladesh Agricultural University, Mymensingh during the period of May to October, 2000 to 2002.

3.1.2.1 Selection of area.

Mymensingh is an important place. The famous Bangladesh Agricultural University (BAU) is situated in this district. Due to this opportunity lot of scientific works are going on in this district and as a result farmers are becoming interested for establishing small scale dairy farms. Milk production is increasing day by day. For this reason various sweetmeat shops are seen in local markets. Some milk processing industries are thinking to setup milk collection centre to collect milk for their processing plant. These are the main reasons for selection of this area. Four

different places in Mymensingh district were selected for this purpose and these were i) Bangladesh Agricultural University Dairy Farm; ii) Mymensingh Town; iii) Local village market and iv) Farmer's house.

3.1.2.2 Collection of milk samples

During experimental period, samples were collected from the four different places and were transferred immediately to the laboratory for analysis.

A total of 120 milk samples were collected taking 30 samples from each of the different sources. Approximately 500 ml of milk were taken for each representative samples. Cleaned pots were used in order to avoid any kind of external contamination.

The following types of milk samples were collected.

a) Samples from BAU dairy farm

b) Samples from Mymensingh town supplied by vendors

c) samples from Local village markets.

d) Samples from farmers house.

3.1.2.3 Parameters studied

The following physical, chemical and microbiological tests were performed with each raw milk samples

3.1.2.3.1 **Physical tests**

a) Organoleptic tests

i) Colour

ii) Flavour

iii) Taste

b) Specific gravity (Sp. gr.)

3.1.2.3.2 **Chemical tests**

a) Acidity content of milk (%)

b) Fat content of milk (g/kg)

c) Solids-not-fat content of milk (g/kg).

d) Total solids content of milk (g/kg).

e) Water content of milk (g/kg).

f) Protein content of milk (g/kg)

g) Lactose content milk (g/kg)

h) Ash content of milk (g/kg).

3.1.2.3.3 Test for defecting adulteration

a) Tests for detecting water added and others (if any)

3.1.2.3.4 Microbiological tests

a) Total viable count/ml of milk

b) Coliform bacterial count /ml of milk

3.1.2.3.4 Analytical Procedure

Organoleptic test was performed visually, lingually and nasally to observe the colour, flavour and taste according to Nelson and Traughat (1964) Appensix-(1).

Specific gravity test was performed by using Quevenne Lactometer, lactometer cylinder and floating dairy thermometer according to the method described by Aggarwala and Sharma (1961).

Fat test was performed by Babcock fat test method described by Eckles *et al.* (1951). Acidity test was done by titrating milk with N/10 NaoH solution by using A.O. A. C (1971) method.

Protein was estimated by formal titration methods. Solids-not-fat (SNF) and Total Solids (TS) content of collected milk samples were performed according to Eckles *et al.* (1951).

The experimental procedures followed for the determination of the number of total viable bacteria in a sample and the detection and enumeration of coliform bacteria were as per method described by American Public Health Association (APHA, 1960).

Lactose was determined by calculation method:

Lactose = Total solids-(Fat + Proteins + Ash).

Detail experimental procedures of above tests are given in the Appendix section.

3.1.2.3.5 Statistical Analysis

Data collected from this experiment were analysed by using Completely Randomized Design (CRD) as per Steel and Torrie (1980). Analysis of variance test was performed to findout the statistical difference within different treatments. In case of significant difference Least Significant Difference (LSD) Test was done to find out the significant difference between treatment means.

RESULTS AND DISCUSSION

A total of one hundred and twenty milk samples collected from four different places were examined to evaluate their qualities, Results obtained from this experiment are presented below.

3.1. 3.1 Physical Parameters

a) Organoleptic test:

i) **Colour:** Out of 120 samples collected from different places of Mymensingh district, 109 samples were (90.67%) golden yellowish white, 11 samples (9.33%) were light yellowish white.

Table 1.1 Average physical parameters of milk samples collected during experimental period.

Physical parameters	Dairy farm	Mymensingh town	Local market	Village farmers
Colour	Golden-yellowish white (30 samples) – 100%	Golden-yellowish white (30 samples) – 100%	Golden-yellowish white (20 samples)=66.67% light golden-yellowish white (10 samples)=33.33%	Golden-yellowish white (26 samples)=86.67% light golden yellowish white (4 samples)=13.33%
Taste	Slightly sweet (30 samples) –100%	Slightly sweet (27 samples)=90% flat (3 sample)=100%	Lightly sweet (21 sample)=70% flat (9 sample)–30%	Slightly sweet (26 sample)=86.67% flat (4 sample) –13.33%
Flavour	Normal (pleasant aromatic) (30 sample) = 100%	Normal (pleasant aromatic) (27 sample)=90% non-milky (3 sample)=10%	Normal (pleasant aromatic) (25 sample)=83.33% (cowy 3 and barney 2) (5 sample) = 16.67%	Normal (pleasant aromatic) (27 sample)=90% non-milky (3 sample)=10%
Sp. Gravity	1.027-1.032	1.017-1.031	1.022-1.032	1.022-1.032
AV.	$1.029^{a}\pm0.00$	$1.027^{b}\pm0.00$	$1.027^{b}\pm0.00$	$1.029^{a}\pm0.00$
Level of significance	**			
LSD value	0.0017			

Differenct superscripts within the same row differ significantly

The colour of all milk samples obtained from BAU dairy farm and Mymensingh town were golden yellowish white (100%) but for Local markets and Village farmers, 66.67% and 86.67% were golden yellowish white respectively. The remaing 33.33% Local market sample showed slightly bluish colour and 13.33% village farmer's samples showed Light golden yellowish colour. Usually the colour of normal cows milk is golden yellowish white due to the presence of fat, casein and small amount of colouring matter (carotene). Eckles *et al.* (1951) stated that milk colour depends upon the breed of animal, the kind of feed consummed and the

amount of fat and solids presence in milk. Lampart (1970) stated the colour of milk varies upon fat, solids-not-fat (SNF) and the size of the fat globles. Samples collected from Bangladesh Agricultural University (BAU) Dairy Farm and Mymensingh Town indicated that their colour were normal (pleasant aromatic). No major abnormalities were detected in the colour of Local markets and village farmers milk although few samples showed slightly golden yellowigh color.

iii) Taste: The taste of milk samples from different sources are shown in (Table 1.1). It was found that out of 120 samples 107 samples (89.33%) were normal in taste (Slightly sweet) and remaining 13 samples (10.67%) were abnormal in taste (flat).

It is evident from the (Table 1.1) that the taste of all milk samples of BAU dairy farm were normal (slightly sweet). But in case of the samples of Mymensingh Town, Local markets and village farmers 90, 70 and 86% respectively showed normal taste. On the other hand, 10, 30 and 14% samples showed flat taste. Slightly sweet taste of milk is due to the presence of lactose (Eckles *et al.* 1951; Judkins and Keener 1960). Flat flavour of milk might be due to low lactose content.

Usually odd taste in the samples might be arises to unhygienic condition where the milking has done, probably the milk for a long time storage prior to sale, which provide opportunity to develop some microorganisms and cause some odd taste like sour, bitter etc. But the results obtained from this study indicated that the flat taste which was deteted from some samples was not due to microbial degradation of milk. In this connection, Judkins and Keener (1960) who reported that milk produced under proper condition had slightly sweet taste.

ii) Flavour: Out of 120 samples 111 samples (90.67%) had normal flavour and 9 samples (7.33%) had abnormal flavour (3 samples were cowy, 2 samples were barny and 6 samples were non-milky in flavour). The flavour of all milk samples collected from BAU dairy farm were normal. The flavour of normal cows milk is pleasant and aromatic. On the other hand, among the samples of another 3 places

namely Mymensingh town, Local market and Village farmers, 90%, 83.33% and 90% had normal flavour respectively.

The differences in flavour of milk may be due to the unhygienic condition during milking or probably some sort of flavoured feed (like bitter weeds, bitter grass, green rye, garlic, onion, silage etc.) consumed by cows during or prior to milking (Olson, 1956), Ward *et al.* (1956). Judkins and Keener (1990) found that flavour of milk produced under sanitary condition was normal. Foley *et al.* (1972) reported that cowy flavour found in milk from cows suffering from ketosis. A barny flavour occurs in the milk of cows housed in poorly ventilated sheds.

Specific Gravity: Specific gravity of milk obtained from different selected places throughout the experimental period are shown in (Table 1.1 and Fig. 1.1). The mean and standard deviation of the specific gravity of milk collected from BAU dairy fram Mymensingh town, Local market and Village farmers were 1.029±0.00, 1.027±0.01, 1.027±0.00 and 1.029±0.00 respectively. Statistically it was found that there were significant differences ($P<0.01$) within the specific gravity of milk collected from different sources.

It was observed that the average specific gravity of milk obtained from BAU dairy farm (1.029±0.00) and Village farmers (1.029±0.00) was significantly higher ($P<0.05$) than that of the specific gravity of milk collected from other two places. We know that the normal range of specific gravity of whole milk is 1.027 to 1.035 with an average of 1.032 (Eckles *et al.* 1951).

From the present study it was observed that specific gravity of milk samples collected from different selected places was within the normal range. Lower specific gravity of milk indicates that the quality of milk is inferior may be due to adulteration of water. Milk fat has some influence on the specific gravity of milk. As the higher the fat content of milk, the lower will be the specific gravity.

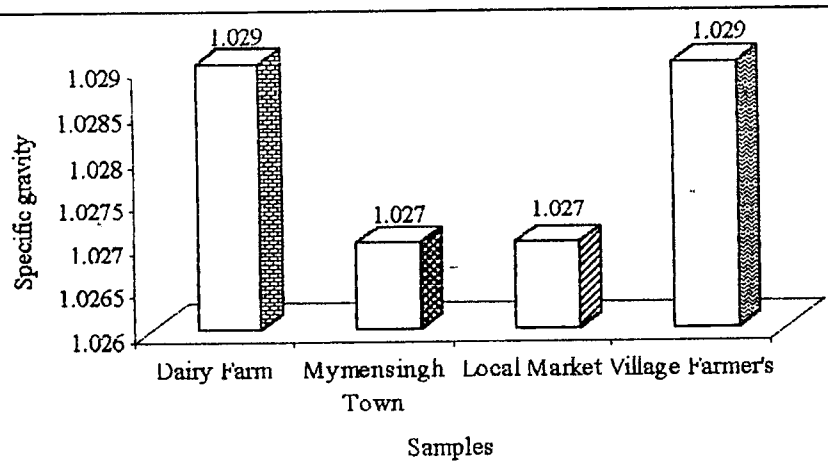

Fig. 1.1 Graph showing the average value of specific gravity of milk in Mymensingh

3.1.3.2 Chemical Parameters

a) Acidity percentage: Results of acidity of raw milk samples collected from BAU dairy farm and other three places were 0.150±0.01, 0.150±0.02, 0.138±0.02 and 0.152±0.01respectively (Table 1.2 and Fig. 1.2). Statistically it was found that there were significant differences ($P<0.01$) within the mean acidity of milk samples collected from different places of Mymensingh district (Appen. table 2). It was also observed that the difference between the highest value (0.179) and the lowest value (0.09) of acidity were (49.72%) in respect of highest value (table 1.3). Generally the acidity of normal milk samples varies within the range of 0.10 to 0.18% within an average of 0.16% (Eckles *et al.* 1951). Judkins and Keenar (1960) reported that the normal acidity of market milk may be ranged from 0.08 to 0.23 per cent. Islam *et al.* (1984) found that the average acidity percentage of cow's milk was 0.15%. The lower acidity of milk may be due to the adulteration of water in milk, which reduces the acidity percentage. The result of acidity test indicated that the values for all samples were within normal range. But slightly lower level of acidity of milk of Mymensingh Town and Local market samples might be due to adulteration with water. The result agrees with the finding of Islam (1984) and Ali (1998).

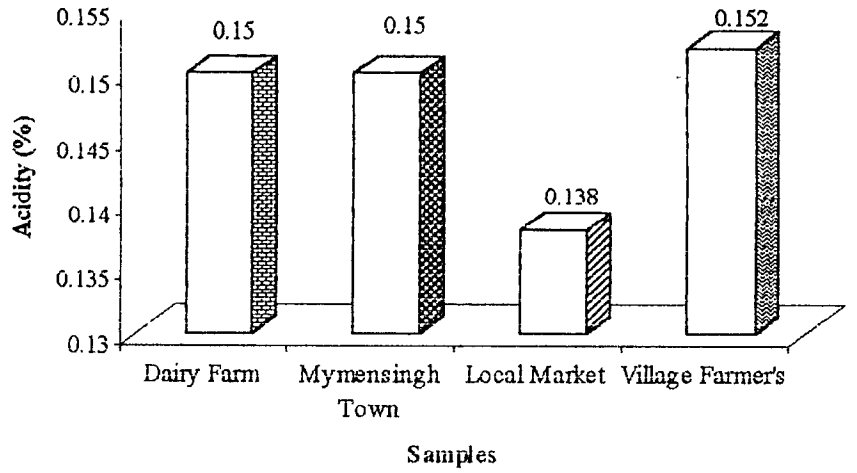

Fig. 1.2 Graph showing the average value of acidity of milk in Mymensingh

b) **Fat content:** The mean and standard deviation of fat content of milk collected from BAU dairy farm, Mymensingh town, Local market and Village Farmers were 46.27±4.48, 33.53±6.48, 35.87±5.42 and 40.3±6.09 (g/kg.) respectively. Statistical analysis showed that the difference between fat content of milk samples collected from the above places were found significant ($P<0.01$). The results are persecuted in (Table 1.2 and Fig 1.3). It was observed that the average value of fat obtained from BAU dairy farm 46.27±4.48 g/kg was higher than the fat content of milk of other places.

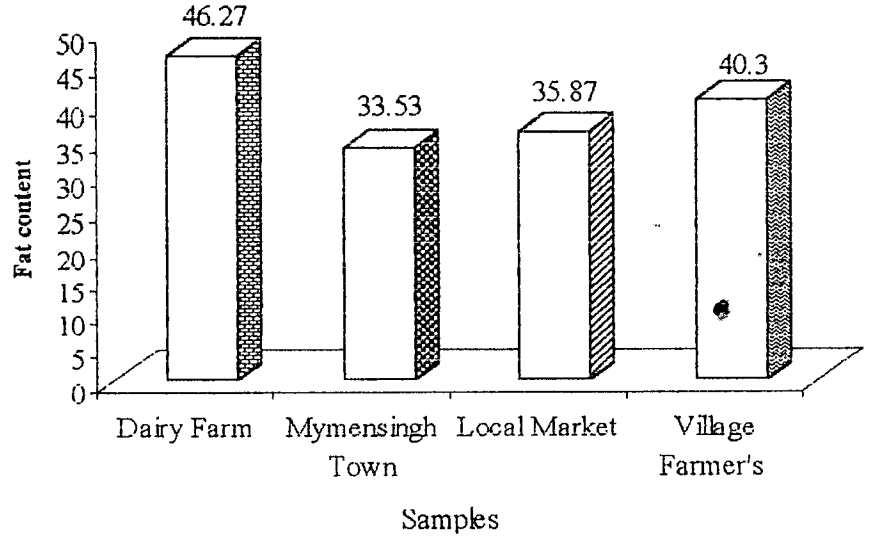

Fig. 1.3 Graph showing the average value of fat content of milk in Mymensingh

According to United states Public Health Service (1965) the minimum standards of milk fat is within the range of 30.0-38.0 gm/kg. The present result showed that the average fat content of the milk samples collected from the above sources were just maintaining the minimum standards of US standards except Bangladesh Agricultural University Mymensingh (BAU) Dairy Farm samples.

The higher fat content of Bangladesh Agricultural University (BAU) Dairy Farm milk indicated that they are maintaining the standards of milk but in case of other samples there might have some sort of adulteration and for that reasons their fat content was lower than Bangladesh Agricultural University (BAU) Dairy Farm milk. The result of this experiment agrees with the finding of Islam (1984) and Alam (1998) who found similar type of results during working with market milk.

c) **Solids-Not Fat (S.N.F) content:** The mean and standard deviation of solids not fat (SNF) content of milk collected from BAU dairy farm Mymensingh town, Local markets and Village farmers were 82.98 ±3.47, 72.18±15.86, 75.73±8.97 and 80.35±6.44 respectively (Table 1.2 and 1.4). The statistical analysis showed that the differences in the solids-not fat (SNF) content of milk samples collected from the four above places were found significant ($P<0.01$) (Appen. Table 4). From the present study it was observed that solids-not-fat (SNF) content of milk samples of Bangladesh Agricultural University (BAU) Dairy Farm and village Farmers milk were within the normal value (8.0-8.5%) recommended by US Public Health Services (1965). But the SNF content of samples of Mymensingh Town and Local markets were slightly below normal indicating that the quality was not upto the mark. Islam *et al.* (1984) showed that the SNF content of milk collected from Local markets was lower than that from the milk collected from BAU dairy farm. This result was in agreement with the present experiment. Alam (1998) obtained the SNF content of milk samples from Aftab fresh raw milk was 8.43%. It can be pointed out that the milk collected from BAU dairy

farm and Village farmers was superior to milk samples collected from Mymensingh town and Local markets.

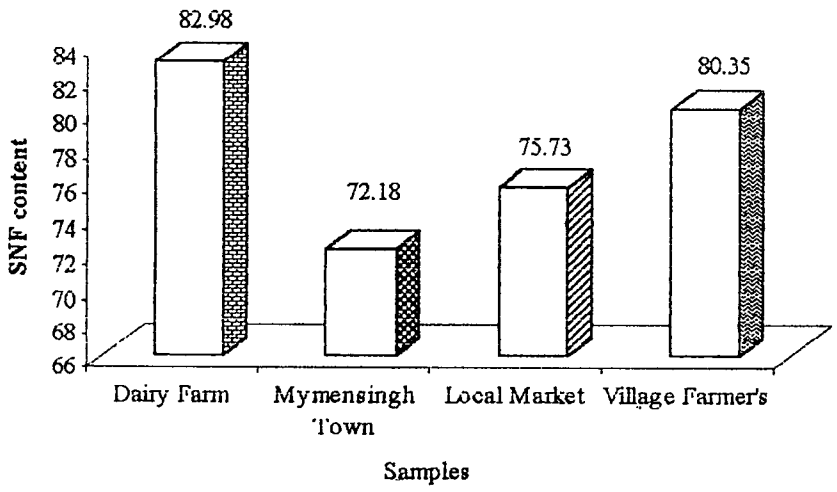

Fig. 1.4 Graph showing the average vavlue of SNF of milk in Mymensingh

d) **Total solids (TS) content:** The average values of total solids content of milk collected from four different selected places are shown in (Table 1.2 and Fig. 1.5). It was observed that the average values of total solids content of milk samples were 128.58±6.86, 104.07±22.75, 111.53±10.49 and 120.72±10.18 for Dairy Farm, Mymensingh Town, Local Market and Village farmer's respectively. Statistical analysis showed that the difference between the total solids (TS) content of milk samples collected from different places were significant ($P<0.01$) (Appen. Table 5). The comparatively lower total solids content of milk collected from Mymensingh town and Local markets than that of BAU dairy farm and Village farmers might be due to the relatively lower fat content of milk as well as solids-not-fat (SNF). Milk collected from Mymensingh town and Local markets could have adultered with water resulting lower fat and SNF percentage, which affected ultimately TS content of milk. The result of this study agrees with the fundings of Islam (1984).

Table 1.2 Summary of the results of chemical parameters of milk collected during experimental period.

Parameters studied	Different places of Mymensingh district				Level of significant	LSD Value
	Dairy farm	Mymensingh town	Local market	Village farmers		
	Mini.-Maxi. Average	Mini.-Maxi. Average	Mini.-Maxi. Average	Mini.-Maxi. Average		
Acidity (%)	0.130-0.165 0.150±0.01	0.09-0.179 0.150±0.02	0.09-0.160 0.138±0.02	0.110-0.160 0.152±0.01	**	0.0098
Fat (g/kg)	35.0-55.0 46.27±4.48	20.0-42.0 33.53±6.48	20.0-48.0 35.87±5.42	30.0-48.0 40.30±6.09	**	3.77
SNF(g/kg)	74.9-89.6 82.98±3.47	46.5-83.3 72.18±15.86	61.0-88.0 75.73±8.97	61.0-89.6 80.35±6.44	**	5.88
T.S (g/kg)	110.1-139.8 128.580±6.86	66.5-120.5 104.07±22.75	91.0-125.4 111.53±10.49	91.0-137.6 120.72±10.18	**	6.75
Water (g/kg)	860.2-888.1 845.51±143.54	879.5-933.5 890.74±18.22	874.6-909.0 888.37±10.55	862.4 –909.0 876.95±15.98	**	8.33
Protein (g/kg)	33.0-37.0 35.10±0.92	32.0-37.0 34.73±1.26	34.0-37.0 35.23±0.77	34.0-37.0 35.43±0.77	*	0.64
Lactose (g/kg)	40.1-46.6 45.95±3.42	8.5-40.9 32.82±9.19	14.1-45.2 33.73±8.59	17.2-44.6 37.70±6.86	**	4.45
Ash (g/kg)	6.5-7.1 6.9±0.21	6.0-7.1 6.6±1.14	5.9-7.1 6.8±0.23	6.8-7.1 6.9±0.1	**	0.12
Water added (%)	No added water	2.0-45.3 12.52±11.83	0.0-35.8 11.04±10.31	0.0-28.2 5.83±7.20	**	5.29

**= Significant at 01% level.
*= Significant at 5% level.

e) **Water content:** Mean and standard deviation of water content of milk samples collected from BAU dairy farm Mymensingh town, Local markets and Village farmers were 845.51±143.54, 890.74±18.22, 888.37±10.55 and 876.95±15.98 ml/L respectively (Table 1.2 and Fig. 1.6). Statistically it was found that there were significant differences ($P<0.01$) within the water content of milk samples collected from the above four different places (Appen. Table 6). The higher water percentage of milk samples of Mymensingh Town and Local markets

indicated that some portion of water might have been added in their samples. The result agrees with the findings of Alam (1998).

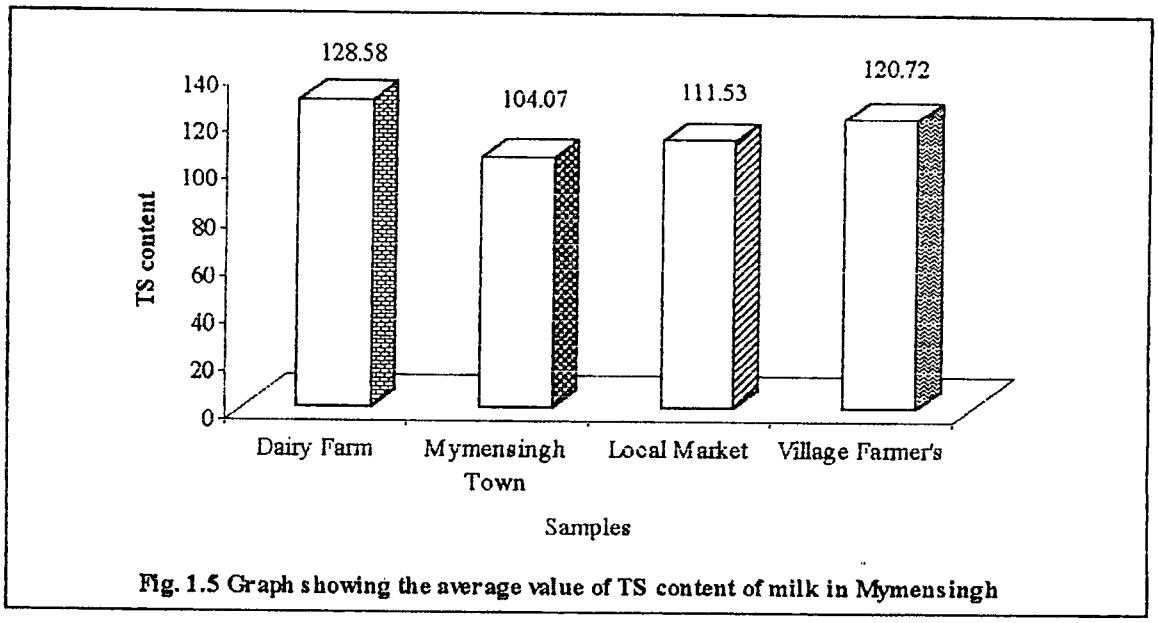

Fig. 1.5 Graph showing the average value of TS content of milk in Mymensingh

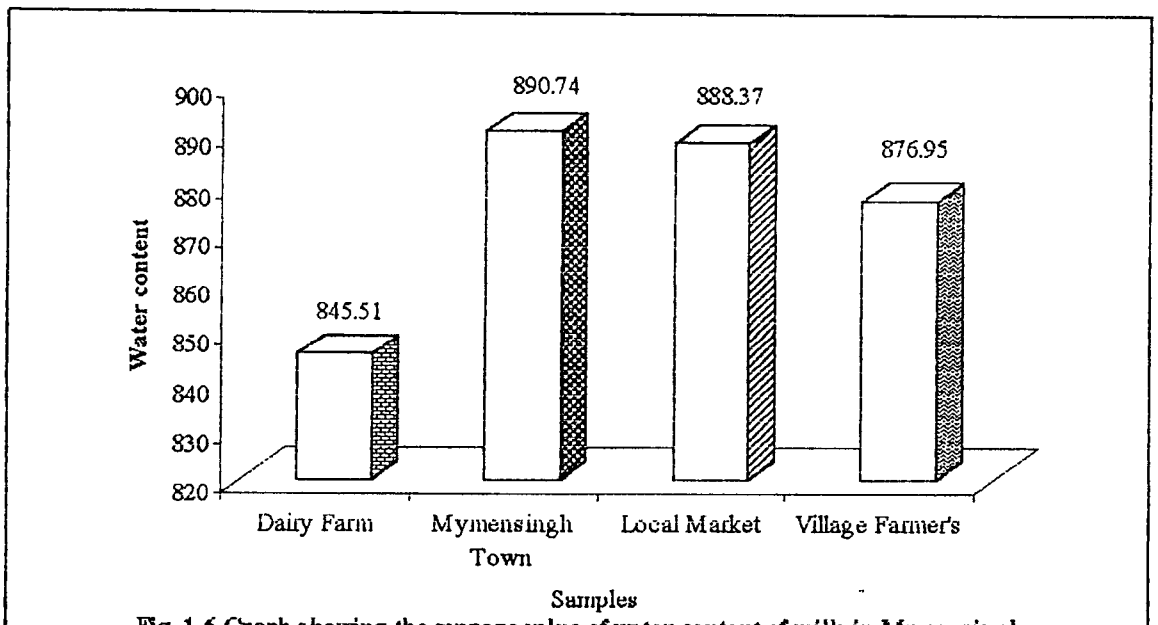

Fig. 1.6 Graph showing the average value of water content of milk in Mymensingh

f) **Protein content:** The mean and standard deviation of protein content of milk samples collected from BAU dairy farm, Mymensingh town, Local markets and Village farmers were 35.10 ±0.92, 34.73±1.26, 35.23±0.77 and 35.43±0.77 g/kg respectively (Table 1.2 and Fig. 1.7). Statistically it was found that there were significant differences ($P<0.05$) within the protein of milk samples collected

from the above four places from Mymensingh district (Appen. Table 7). The result agrees with the work of Filiptovic (1953) and Overman *et al.* (1953) whom reported that the average values of protein were 33.3 gm/kg and 35.2 g/kg respectively for their samples. In another experiment Ali (1991) found that the average value of protein of milk samples from BAU dairy farm, different Hall milk suppliers and vendors were 33.2±19, 33.5±0.03 and 33.1±0.16 g/kg respectively.

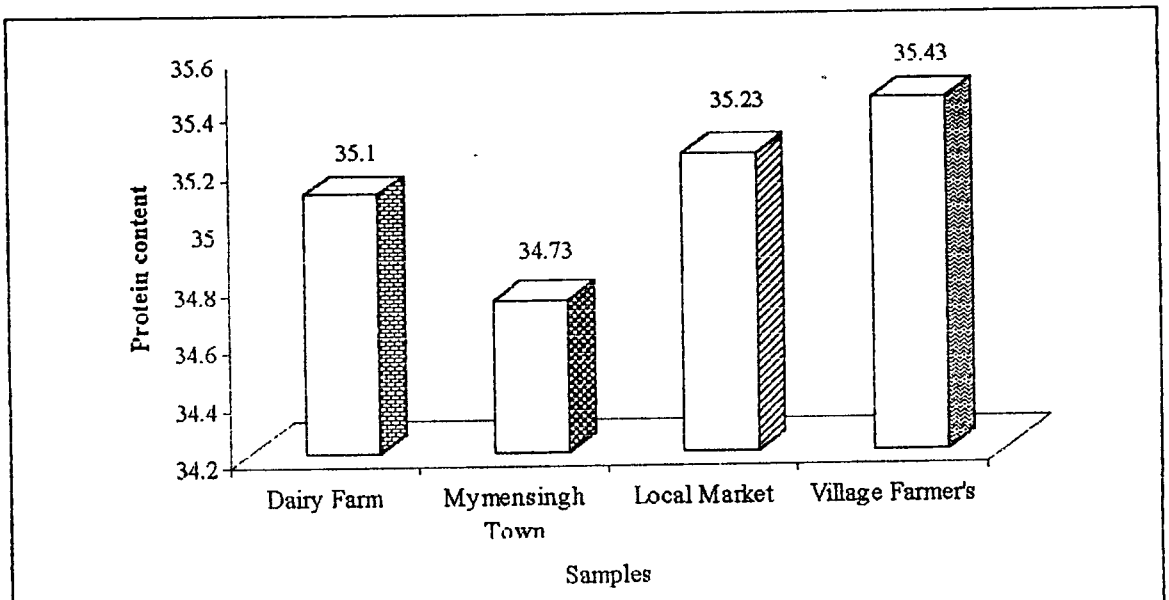

Fig. 1.7 Graph sowing the average value of protein content of milk in Mymensingh

g) **Lactose content:** The mean and standard deviation of lactose content of milk samples collected from BAU dairy farm Mymensingh town, Local markets and Village farmers were 40.957±3.42, 32.82±9.19, 33.73±8.59 and 37.70±6.86 respectively (Table 1.2 and Fig. 1.8). Statistically it was found that there were significant differences ($p<0.01$) within the lactose of milk collected from different sources (Appen. Table 8). Generally milk contains 4.7 to 4.9% lactose (Jennes and Patton, 1959). From the present study it was observed that lactose content of milk obtained from Mymensingh town and local markets was lower than the milk samples collected from BAU dairy farm and Village farmers. That might be due to addition of water in milk which decreases the lactose conent.

Addition of water might have diluted the lactose content of milk. Ali (1998) found that the average lactose of milk samples from BAU dairy farm, different Hall milk suppliers and vendors were 45.0±0.13, 40.0±0.18 and 38.7±0.19 gm/kg respectively.

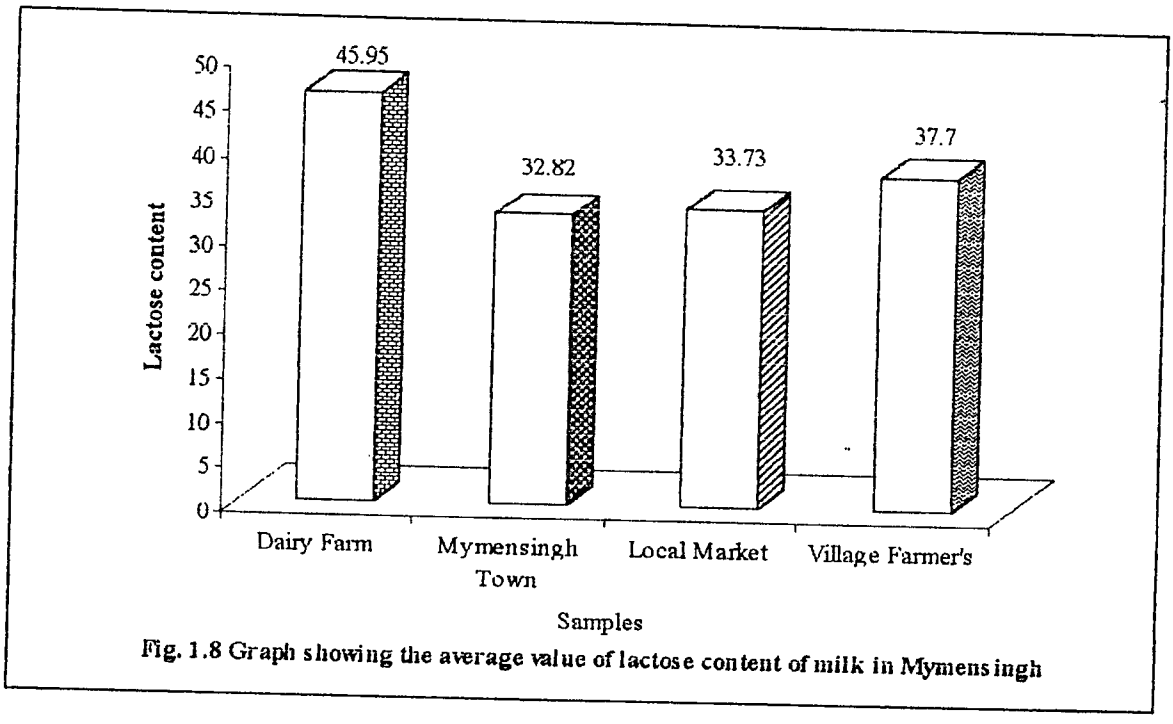

Fig. 1.8 Graph showing the average value of lactose content of milk in Mymensingh

h) **Ash Content:** The mean and standard deviation of milk samples collected from BAU dairy farm Mymensingh town, Local markets and Village farmers were 6.9±0.21, 6.6±1.14, 6.8±0.23 and 6.9±0.10 respectively (Table 1.2 and Fig. 1.9). Statistically it was found that there were significant differences ($P<0.01$) within the ash content of milk collected from the above sources (Appen. Table 9). The lower content of ash might be due to adulteration of milk with water. Ash content of cows milk ranges from 0.06 to 0.80% with an average value of 0.65%. This result agrees with the findings of Eckles, *et al.* (1929).

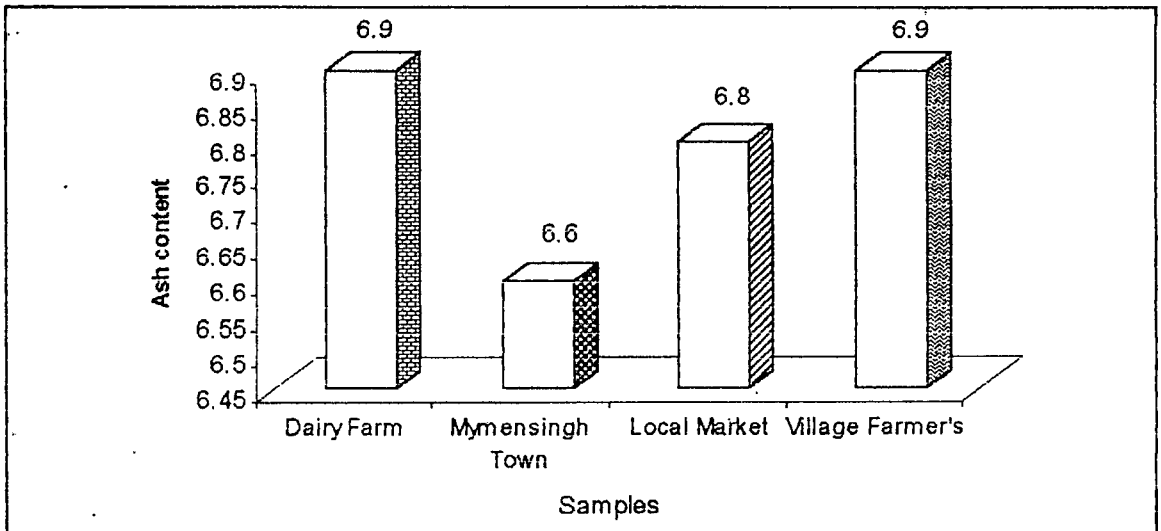

Fig. 1.9 Graph showing the average value of ash content of milk in Mymensingh

3.1.3.3 Tests for detecting adulteration

a) Percent of water added: The mean and standard deviation of percent of water added in milk collected from BAU dairy farm Mymensingh town, Local markets and Village farmers were 3.08±3.22, 12.52±11.83, 11.04±10.31 and 5.83±7.20 respectively (Table 1.2 and Fig. 1.10). It was obtained that the average percent of water added in BAU dairy farm milk was lower than that of milk samples collected from Mymensingh town and Local markets which were 12.52±11.83 and 11.04±10.31 respectively. The percent of water added of milk obtained from Village farmers was also found lower than that of market and town milk. This is due to special attention was taken by the researcher when collect milk from the BAU dairy farm and Village farmers and some direction was given to them to avoid unfair means when/ after milking. The percent of water added also affect the other constituents of milk. Statistical analysis showed that the difference between the percentage of water added of milk samples was found significant ($P<0.01$) (Appen. table 10). It was also observed that the difference between the highest value (45.3) and the lowest value (0.00) of percent of water added were 100% in respect of highest value (table 1.3). The milk samples collected from Mymensingh town, Local markets and Village farmers may be adulterated with

specific adulterants like (sugar and flour added and preservatives like H_2O_2 Formalin and $NaHCO_3$) were tested in the Laboratory. But no adulterants were found out of 150 samples from the above sources. Statistical analysis showed that the differences between the percent of water added of milk samples collected from different four places was found significant ($P<0.01$).

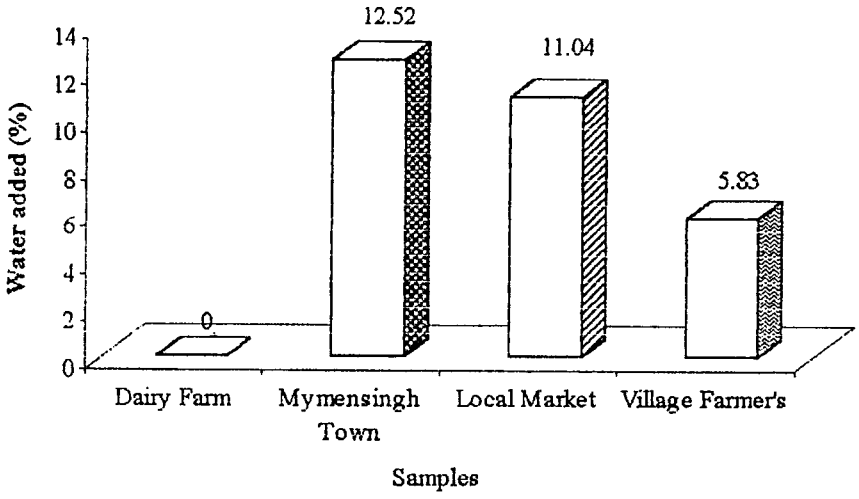

Fig. 1.10 Graph showing the average value of percent of water added of milk in Mymensingh

3.1.3.4 Microbiological Parameters

a) **Total Viable Bacterial Count:** The average values of total viable bacterial count/ml. of milk samples collected from BAU dairy farm, Mymensingh town, Local markets and Village farmers were 42430 CFU/ml (log 4.627), 38733 CFU/ml (log 4.588), 82.367 CFU/ml (log 4.915) and 109570 CFU/ml (log 5.039) respectively (Table 1.3 and Fig. 1.11). Statistically it was found that there were significant differences ($P<0.01$) within the total viable bacterial count of milk samples collected from four different sources. (Appen. Table 11). It was observed that the average value of total viable bacteria obtained from Village farmers (109570 CFU/ml) was significantly higher ($p<0.01$) that other milk samples. According to American Public Health Association, (1960), average standard plate count for 'grade A' raw milk to pasteurized will not exceed 200000/ml. The result indicated that bacterial count of different milk samples

were within normal range. collected from four different places were "Grade A'' category. Total bacterial count/ ml of milk may be depends mainly on maintaining proper hygienic condition.

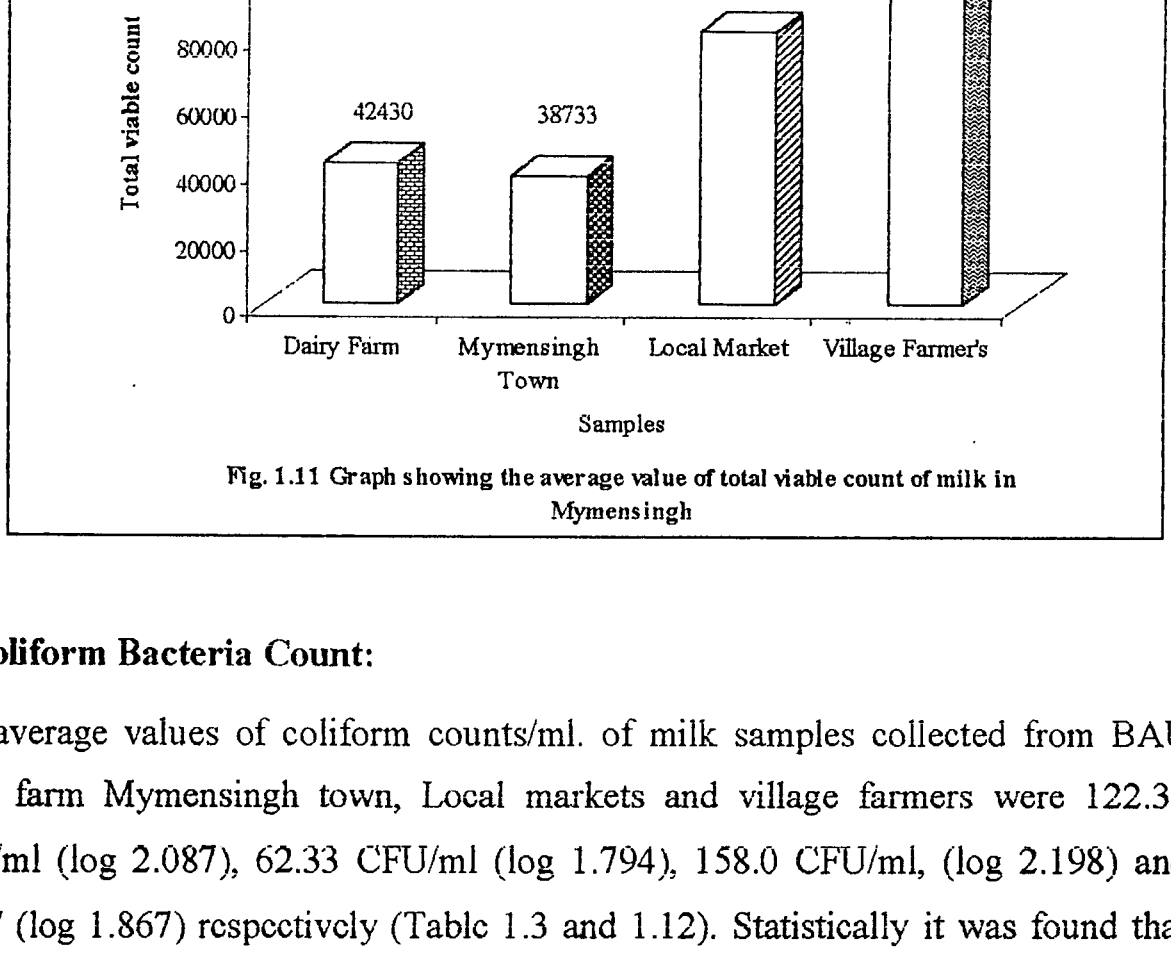

Fig. 1.11 Graph showing the average value of total viable count of milk in Mymensingh

b) Coliform Bacteria Count:

The average values of coliform counts/ml. of milk samples collected from BAU dairy farm Mymensingh town, Local markets and village farmers were 122.33 CFU/ml (log 2.087), 62.33 CFU/ml (log 1.794), 158.0 CFU/ml, (log 2.198) and 73.67 (log 1.867) respectively (Table 1.3 and 1.12). Statistically it was found that there were significant differences ($P<0.01$) within the coliform bacteria of milk samples collected from different four places of Mymensingh district (Appen. table 12). It was also observed that the highest value (200 CFU/ml) and the lowest value (20 CFU /ml) of coliform bacteria count per ml were 90% in respect of highest value (table 1.5). It was observed that the average values of coliform from Local markets (158 CFU/ml) was significantly higher ($P<0.01$) and evening milk of BAU dairy farm (46.33 CFU/ml) was significantly lower than the coliform bacteria of

milk samples collected from other places. Fresh milk samples contain not more than 10 per ml or 100 CFU/ml coliform bacteria, which is a very lenient standard (Foster *et al.* 1958). Kantona et al. (1982) showed that cows kept and milked using proerly cleaned and disinfected equipment contain less than 01 coliform /ml of milk, where as cows milked with improperly cleaned equipment contain 2400 coliform. Coliform bacteria are one of the major indications of hygienic condition of milk. Less number of coliform bacteria /ml of hygienically produced milk indicated that the sources of coliform organisms, like contamination of milk from cow-dung, urine, cows udder, milk container, soil, dust were avoided in the hygienic condition.

Therefore, it could be concluded that the improvement of hygienic condition during milk is important to reduce the coliform bacteria in milk.

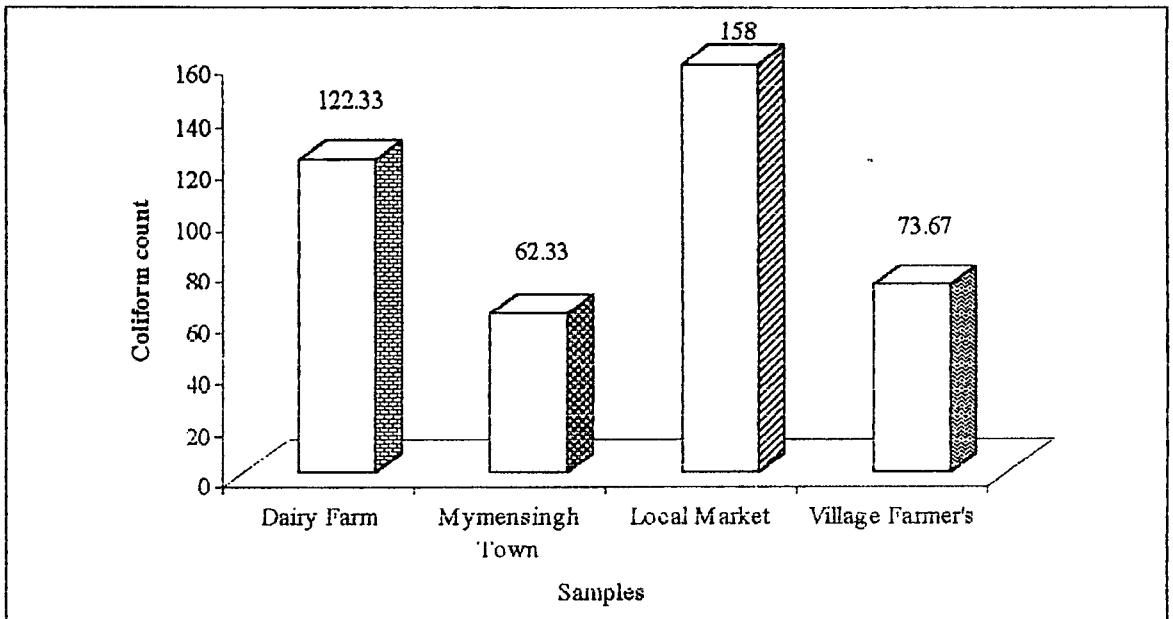

Fig. 1.12 Graph showing the average value of coliform count of milk in Mymensingh

Table 1.3 Average total viable count and coliform count in raw milk during experimental period.

Sources of milk	No. of samples analysed	Total viable count /ml.		Coliform count /ml		Level of significant	LSD value
		Cfu/ml	log	cfu/ml	log		
Dairy Farm	30	42430	4.627	122.33	2.087		TVC=14.97
Mymensingh Town	30	38733	4.588	62.33	1.794		Caliform count 1.35
Local Market	30	82367	4.915	158.0	2.198	**	
Village Farmers.	30	109570	5.039	73.67	1.867		

All counts are expressed in logarithms

** = Significant at 1 % level.

Table 1.4 Summary of the results of total viable and coliform bacteria per ml of 150 milk samples.

Parameters	Minimum values	Maximum values	Mean	Standard Deviation	% difference
Viable bacteria per ml (x1000)	4.0	120.0	62.440	36.385	96.67
Coliform bacteria per/ml (x 10)	2.0	20.0	9.253	4.619	90

SUMMARY AND CONCLUSION

The experiment was conducted at Dairy Technology amd Microbiology Laboratory of the Department of Dairy Science, Bangladesh Agricultural University, Mymensingh to evaluate the physical, chemical and microbiological qualities of milk samples collected from four different selected places in Mymensingh district.

After collection of milk samples from that place, they were taken immediately to the laboratory for analysis. The experiment was conducted for a period of six months starting from May to October, 2001- 2002.

The parameters used to monitor the physical, chemical and microbiological qualities of milk samples were as follows:

1. **Physical tests:** colour, taste, flavour and specific gravity of milk samples
2. **Chemical tests:** acidity, fat, solids- not- fat (SNF), total solids (TS), water content, protein, lactose, ash and detecting percent of water added
3. **Microbiological tests:** total viable bacteria count and coliform bacteria count.

From the organoleptic test it was observed that out of 150 samples of different places of Mymensingh district, 136 samples 90.67 percent were normal in colour i.e. yellowish white and14 samples 9.33 percent were light yellowish white. There were no marked difference of colour observed among the raw milk samples. In case of taste, out of 150 samples 134 samples 89.33 percent were normal in taste (slightly sweet) and remaining 10.67 percent were abnormal in taste (flat) . In respect of flavour, 139 samples 90.67 percent had normal flavour (pleasant and aromatic) and remaining 11 samples 7.33 percent were abnormal flavour (3 samples were cowy, 2 samples were barly and 6 samples were non-milky in flavour. The average specific gravity of collected ted milk samples from BAU dairy farm (morning and evening), Mymensingh town, Local markets and village farmers were 1.029± 0.00, 1.029± 0.00, 1.027 ±0.00, 1.027±0.00 and1.029±0.00 respectively. The specific gravity of milk samples collected from the above places was within the normal range. Milk fat has some influence on the specific gravity. Lower specific gravity of milk indicates that the quality of milk is inferior may be due to adulteration of water.

From chemical analysis it was observed that the average content of fat, solids-not-fat, total solids, protein, lactose and ash gm/kg obtained from BAU dairy farm Mymensingh town, local markets and village farmers were 46.27± 4.48,

33.53±6.64, 35.87±5.42, 40.3±6.09; 82.98±3.47, 72.184±15.86, 75.73±8.97, 80.35±6.44; 128580±6.86, 104.07±22.75, 111.530±10.49, 120.712±10.18; 35.10±0.92, 34.73±1.26, 35.23±0.77, 35.43±077; 40.95±3.42, 32.82±9.19, 33.73±8.59, 37.7±6.86; 6.9±0.21, 6.6±1.14, 6.8±0.23, 6.9±0.01 respectively. The average water content of milk samples collected from the above places were 845.509±143.45, 890.743±18.22, 888.370±10.55, 876.950±15.98 (ml/L) respectively. The acidity percent and the percent of water added in the same order were 0.150±0.01, 0.150±0.02, 0.138±0.02, 0.152±0.01and 3.08±3.22, 3.69+2.88, 12.52+11.83, 11.045+10.31, 5.83+7.20 respectively. Statistical analysis showed that the different parameters of milk samples among the four different places were significant ($P<0.01$) except the different of water ml/L) and lactose gm/kg were significant ($P<0.05$). It was observed that the average values of total viable bacteria obtained from village farmers (109570 CFU /ml) was significantly higher ($P<0.01$) and Mymensingh town (38733 CFU/ml) milk were significantly lower than the total viable bacteria of milk of other samples. Total bacterial count/ml of milk may be depends mainly on maintaing proper hygienic condition. From coliform counts it was observed that coliform bacteria are one of the major indications of hygienic condition of milk. Higher bacterial content indicate that the milk may be produced in unhygienic condition or milk may be contaminated. From the results obtained it may be concluded that to get pure and wholesome milk the producers and distributors must be honest, the environment of barn must be hygienic, milking room must be well ventilated, the equipments must be clean and sanitized, cows must be healthy and free from disease and supplied balanced ration and pure water to the cows. For the production of better quality milk in the rural condition for better hygienic milk condition to reduce the incidence of bacteria for better quality of milk in terms of keeping quality and public health.

Experiment 2 Studies on the quality of market milk collected from some selected areas of Northern part of Bangladesh

INTRODUCTION

This is the second experiment of the series of studies in which attempts were made to monitor the quality of market milk available in northern part of the country. Northern part of Bangladesh is a very important milk pocket area where large number of cross-bred dairy cows are reared by farmers by using co-operative district of northern area of the country produces huge amount of milk and Bangladesh Milk Producers Co-operative Union LTd (Milk-vita) has set up a big milk processing plant and collecting milk from the farmers through different societies. Milk-vita is grinning technical and financial support to the farmers for rearing dairy cows. As a result milk production of that area is increasing day by day. Milk-vita is processing about Two Lacks litre of milk every day through their Sirajgong Milk Processing Plant, which is situated at Baghabarishat of that district.

In addition to that milk processing plant, Milk-vita has set up some milk chilling centre, whore milk is collected and stored. There after collected milk is delivered to the processing plant. Due to huge production of milk in that areas, lot of Non-government Organization (NGO's) have also set up milk collection centre. Pabna is another import district of northern part where famous Pabna cows are available and milk production of the area is also high.

Although a huge amount of milk is producing in northern part of Bangladesh but very limited research works have been in the part to monitor the quality of market milk of that areas. For this reason this attempt was made to evaluate the quality of milk available in northern part of Bangladesh.

Materials and Methods

The experiment was conducted at Dairy Technology and Microbiology Laboratory of the Department of Dairy Science, Bangladesh Agricultural University, Mymensingh, during the period of May to October 2001- 2002. In addition to Bangladesh Agricultural University (BAU) Dairy Technology and Microbiology Laboratory, some analytical works were also done in the (i) Quality control laboratory of Bangladesh Milk. Producers C0-operative Union Ltd. named as Milk-Vita, Baghabarighat, Sirajgonj and (ii) Livestock disease investigation centre Sirajgonj under the Ministry of Livestock and Fisheries.

3.2.2.1 Selection of area

Sirajgonj in greater Pabna district is a place where the average production of milk is very high. The main reason for that is the presence of more Pabna cows and cross-bred cows in that area which produces more milk than our local dairy cows. So more fluid milk are available in that area. The milk producers sell their milk in open local market, goalas also supply milk in Milk-Vita through different societies. Pabna Ghee is famous throughout the country, as because more milk is available in the area. For this reason, Bangladesh Milk Producers Co-operative Union Ltd. already established a milk processing plant (Milk-Vita) at Bnghabarighat in Sirajgonj, Bangladesh Rural Advancement Committee (BRAC) and other organizations also established chilling centres in that area and collecting milk through societies. There is a great opportunity to set small dairy industry in future to serve the demand of increasing milk productions and demand of the population. Communication facilities with Dhaka to Sirajgonj and other district is very good. For this reason, this area was selected for experimental purpose.

3.2.2.2 Collection of milk samples

During experimental period, samples were collected from the four different areas by keeping them in an icebox and were transferred to the laboratory for analysis.

The areas were

a) samples from Sirajgonj town supplied by vendors

b) Samples from Local village markets of Sirajgonj

c) samples from Local village markets of Pabna

d) Samples from society of milk shed area of Pabna.

A total of 80 milk samples were collected having 20 samples from each of the mentioned areas. The parameters used to monitor the quality of milk were almost similar to the first experiment.

The experimental procedures of all physical, chemical and microbiological parameters were similar to the first experiment.

Statistical analytical procedure was also similar to the first experiment.

RESULTS AND DISCUSSION

3.2.3.1 Physical Parameters

The physical parameters were studied after receiving milk from four different places of Sirajgonj and Pabna district. The physical parameters mainly studied were organoleptic tests (colour, taste and flavour) and the specific gravity of milk samples.

a) Organoleptic tests: The colour, taste and flavour of the milk samples collected from different places were normal i.e. golden yellowish white, slightly sweet and mild aromatic (Table 2.1). Only three samples of Sirajgong Town milk showed flat taste. In this experiment, no marked differences were observed among the milk samples regarding colour, taste and flavour of milk collected from four different places of Sirajgonj and Pabna district. The colour, taste and flavour of milk varies depends on many factors which has been discussed in the previous experiments.

b) Specific gravity: Specific gravity of milk obtained from four different places through out the experimental period is shown in (Table 2.1 and Fig. 2.1). The mean and standard deviation of specific gravity of milk samples collected from

Sirajgonj town, Local market of Sirajgonj, Village market of Pabna and milk shed area of Pabna (society) were 1.029± 0.0, 1.027±0.0, 1.029 ±0.0 and 1.027±0.0 (Table 2.1) respectively. Statistically it was found that there were significant differences (P<0.05) within the specific gravity of milk collected from the above four places (Appen. Table 14). The specific gravity of milk obtained from Sirajgonj town (1.029±0.00) and Village market of Pabna (1.029+0.00) was significantly higher (P<0.05) than that of the specific gravity of milk collected from other two places. It was observed that the differences between the highest value (1.033) and lowest value (1.017) of specific gravity were 1.54% in respect of highest value (Table 2.3)

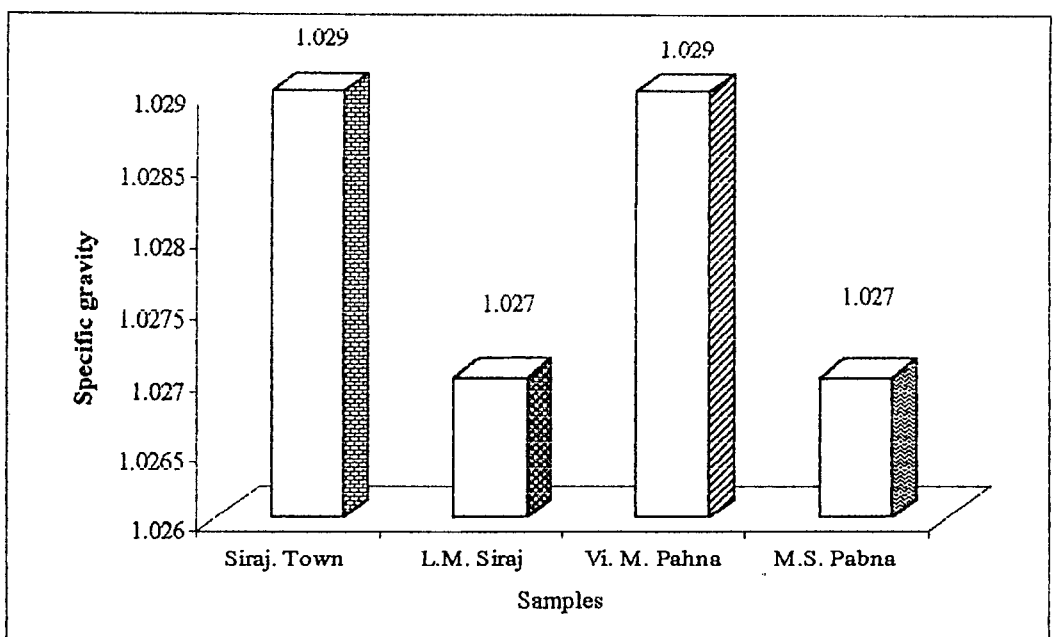

Fig. 2.1 Graph showing the average value of specific gravity of milk in northern part of Bangladesh

From the study it was found that the specific gravity of milk samples collected from the above places was within the normal range. This indicates that quality of collected milk samples were good.

Table 2.1 Average physical parameters of milk samples collected during experimental period.

Physical parameters	Sirajgonj town	Local market of Sirajgonj	Village market of Pabna	Milk shed area of Pabna (society)
Colour	Golden yellowish white 20 samples =100%	Golden yellowish White 20 samples =100%	Golden yellowish white 20 samples =100%	Golden yellowish white 2o samples =100%
Taste	Slightly sweet 17 Samples =85%, flat of 3 samples =15%	Slightly sweet 20 samples −100%	Slightly sweet 20 samples = 100%	Slightly sweet 20 samples − 100%
Flavour	Normal 20 samples =100%	Normal 20 samples =100%	Normal 20 samples =100%	Normal 20 Samples −100%
Sp. Gravity	1.017-1.033 $1.029^{a}\pm0.0$	1.026-1.029 $1.027^{b}\pm0.0$	1.027-1.030 $1.029^{a}\pm0.0$	1.026-1.030 $1.027^{b}\pm0.0$
Level of signaticant	*			

* =Significant at 5% level

Different superscripts between two means indicates significant difference

3.2.3.2 Chemical Parameters

a) Acidity Percentage: The average acidity percentage of milk samples collected from Sirajgonj town, Local market of Sirajgonj, Village market of Pabna and milk shed area (Society) of Pabna were 0.154±0.01, 0.148±0.0, 0.151±0.0 and 0.152±0.01 respectively (Table 2.2 and Fig. 2.2) statistically it was found that there were no significant differences within the mean acidity of milk samples collected from the above four places (Appendix-1). It was also observed that the differences between the highest value (0.168) and the lowest value (0.127) of acidity were 24.4% in respect of highest value (Table 3). In our experiment the acidity

percentage of milk samples was within the normal range of acidity which indicated that all the milk samples were fresh during analysis in the laboratory and values agreed with the results of several researchers. Nakac *et al* (1978) reported that the average percentage of titratable acidity of cows milk was 0.14%. Ali (1998) also found the similar results of acidity.

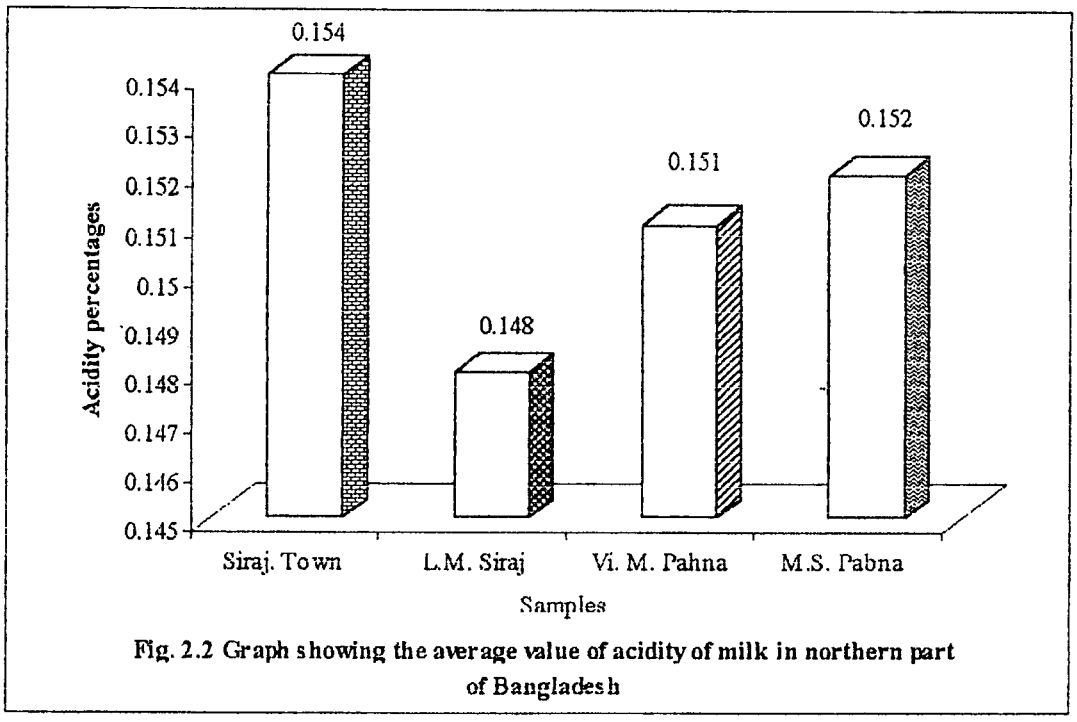

Fig. 2.2 Graph showing the average value of acidity of milk in northern part of Bangladesh

b) Fat content: The average values and standard deviation of fat content of milk samples collected from four different places were 31.50±4.97, 37.10 ±2.27, 43.25±3.10 and 43.25±2.07 (g/kg) respectively (Table 2.2 and Fig. 2.3). Statistical analysis showed that the differences between fat content of milk samples collected from different places were found significant ($P<0.01$) (Appen. Table 15). It was also observed that the difference between the highest value (49.0 g/.kg) and the lowest value (20.0 g/kg) of fat was 59.2% in respect of highest value (Table 2.3). It was found that the average value of fat obtained from village market of Pabna and milk shed area (society) of Pabna (43.25 ±3.10 and 43.25±2.07) was highest than the fat content of milk samples collected from other two places of Sirajgonj district. Ghani and Raman (1954) observed that the average content of fat in native cows

milk was 5.4% (54 g/kg). It appears that the fat content of indigenous cows are higher than the cross-bred cows. Yadav and Sarawat (1982) showed that fat content of milk obtained from local markets of Varanasi town was lower than the fat content of control samples. In another experiment, Mishra and Nayak (1962) found that fat content in milk of indigenous cows in Orisa was 46.9 g/kg. Hossain (1968) showed that the fat content of indigenous cow's milk was 46.0±6.64 g/kg). According to US Public Health Service (1965) milk should contain minimum 3.5% (35 g/kg) butter fat.

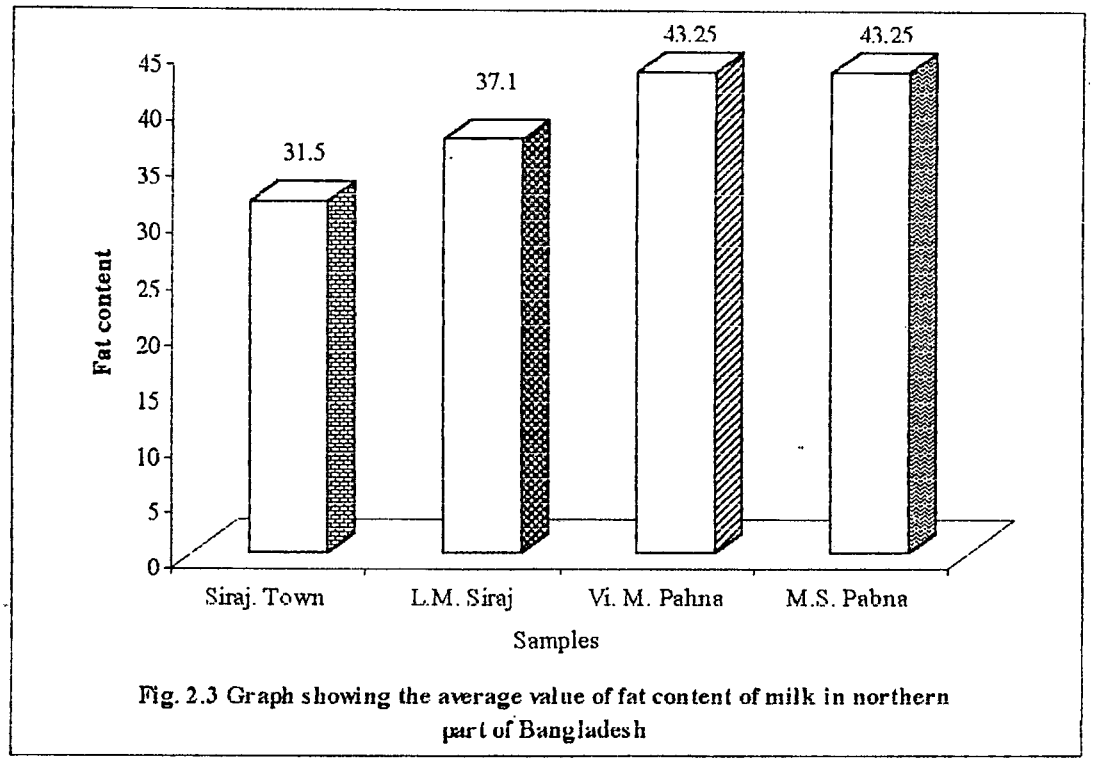

Fig. 2.3 Graph showing the average value of fat content of milk in northern part of Bangladesh

Table 2.2 Summary of the results of chemical parameters of milk collected during experimental period.

Parameters studied	Different places of Pabna/Sirajgonj district					
	Sirajgonj town	Local market of Sirajgonj	Village market of Pabna	Milk shed area(Society) Pabna	Level of Single	LSD value
	Mini-Max. Average	Mini-Max. Average	Mini-Max. Average	Mini-Max. Average		
Acidity (%)	0.127-0.168	0.143-0.153	0.143-0.163	0.143-0.163	NS	-
	0.154±0.01	0.148±0.00	0.151±0.00	0.152±0.01		
Fat (g/kg)	20.0-40.0	33.0-40.0	40.0-46.0	37.0-49.0		2.21
	31.50±4.97	37.10±2.27	43.25±3.10	43.25±2.07	**	
SNF (g/kg)	46.5-90.5	72.0-79.1	76.7-85.3	73.0-81.3		3.02
	78.05±11.32	75.17±1.96	80.90±2.37	77.27±2.41	*	
TS (g/kg)	66.5-130.5	107.0-115.6	120.5-128.8	113.0-125.0		5.30
	109.56±15.23	112.26±2.01	124.15±2.62	120.54±3.49	**	
Water (g/kg)	869.5-933.5	884.4-893.0	871.2-882.0	874.7-887.0		5.30
	890.47±15.22	887.74±2.01	875.95±2.61	879.44±3.47	**	
Water added (%)	0-45.3	6.9-15.3	0.0-9.7	4.3-14.1		3.34
	9.19±12.39	11.53±2.31	4.78±2.75	9.05±2.83.	*	

**= Significant at 1% level.
*= Significant at 5% level
NS= Non-significant.

Table 2.3 Summary of the results of physical and chemical parameters of milk samples in Northern area of Bangladesh.

Parameters	Minimum values	Maximum values	Mean	Standard deviation	% difference
Specific gravity	1.017	1.033	1.028	0.002	1.54
Acidity (%)	0.127	0.168	0.151	0.026	24.4
Fat g/kg	20.0	49.0	3.87	5.9	59.2
S.N.F. g/kg	46.5	90.5	77.8	6.2	48.6
Total soilds g/kg	66.5	130.5	116.6	9.8	49.0
Water g/kg	869.5	933.5	833.4	9.8	6.8
% watcr addcd	0.00	45.3	8.6	6.9	100

c) Solids-not-fat (SNF) content: The average value and standard deviation of milk samples collected from four different places were 78.05±11.32, 75.17±1.96, 80.90±2.37 and 77.27±2.41 (g/kg) respectively (Table 2.2 and Fig. 2.5). The statistical analysis showed that the differences between the SNF content of milk samples collected from four place found significant ($P<0.05$) (Appen. Table 16). It was also observed that the difference between the highest value (90.5 g/kg) and lowest value (46.5 g/kg) were 48.6% in respect of highest value (Table 2.3). SNF content of all milk samples were within the normal range (8.0-8.5%/ g/kg) recommended by US Public Heath Service (1965). This indicates that the quality of milk samples collected from different places was not upto the mark. Yadab and Saraswat (1982) in an experiment found that SNF content varies from 6.39% to 8.86% or 63.9 to 88.6 g/kg which agrees with these findings. Yoshida (1969) found lower SNF content in the market milk in Fukuyama, Japan.

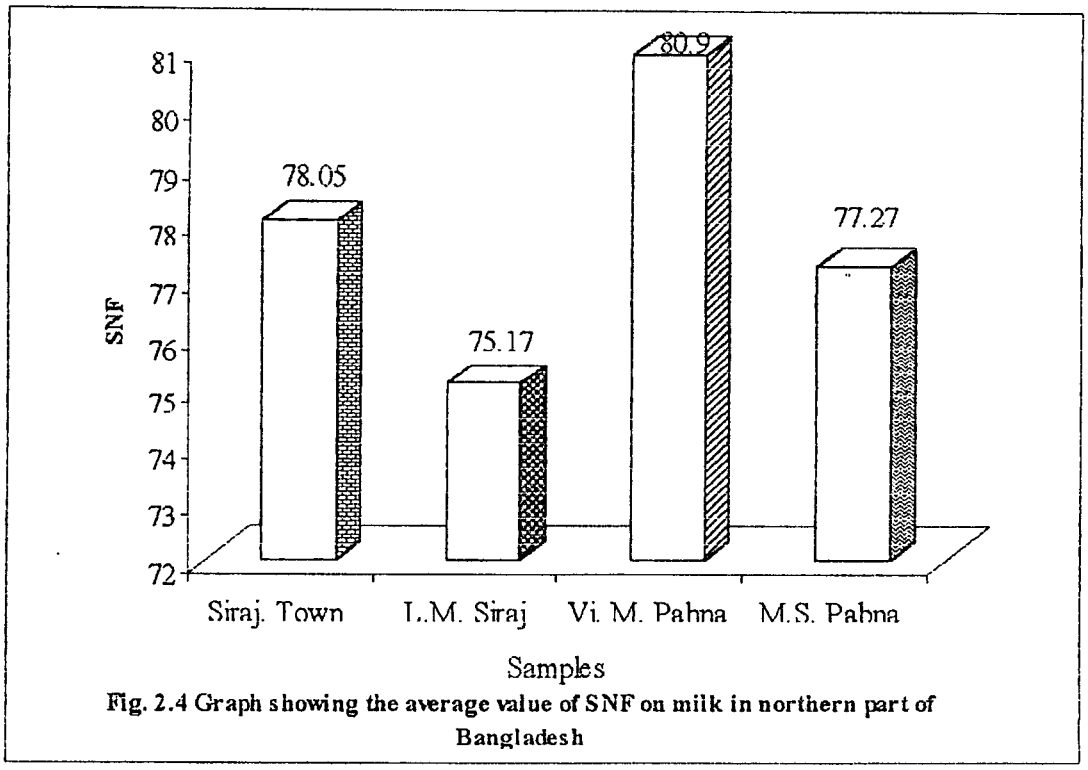

Fig. 2.4 Graph showing the average value of SNF on milk in northern part of Bangladesh

e) **Total solids (TS) content:** The average values of total solids content of milk Samples collected from four different selected places were 109.56±15.23, 112.26±2.01, 124.15±2.62 and 120.54±349 respectively

(Table 2.2 and Fig. 2.5). Statistical analysis showed that the difference between the total solids (TS) content of milk samples collected from four places were significant ($P<0.01$). It was also observed that the difference between the highest value (130.50 g/kg) and lowest value (66.50 g/kg) of total solids (TS) were 49.0% in respect of highest value. The comparatively lower total solids (TS) content of milk collected from Sirajgonj town may be due to the relatively lower content of fat as well as SNF.

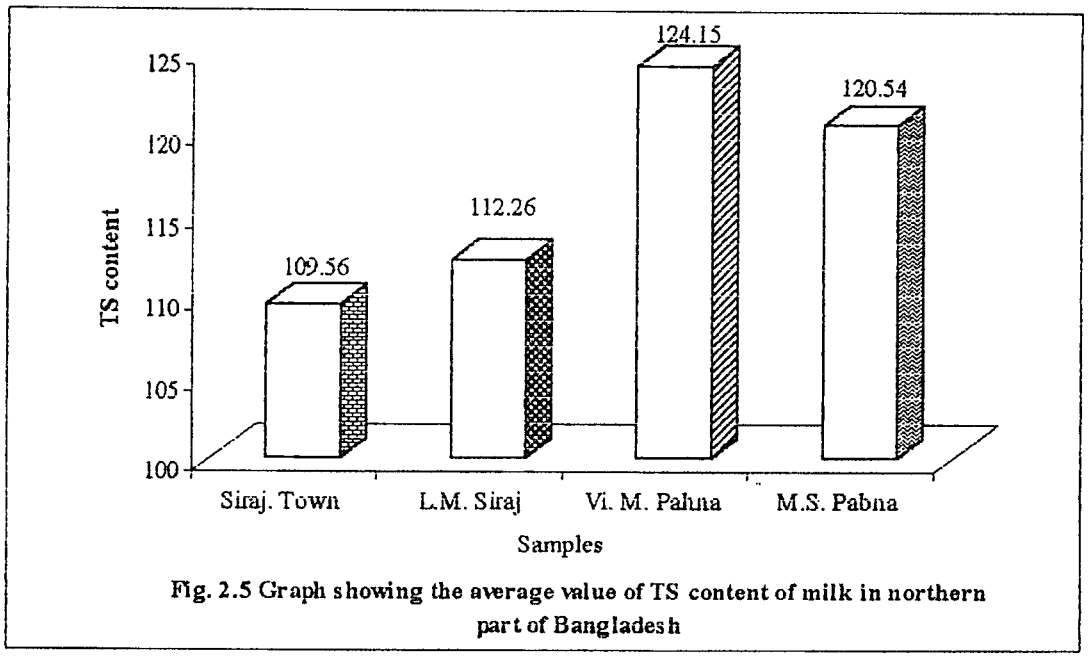

Fig. 2.5 Graph showing the average value of TS content of milk in northern part of Bangladesh

f) **Water content:** The average values and standard deviations of water content of milk samples collected from four different places were 890.47±15.22, 887.74±2.01, 875.95±2.61 and 879.44±3.47 (g/kg) respectively (Table 2.2 and Fig. 2.6). Statistical analysis showed that the differences between the water content among the four places were found significant ($P<0.01$). It was also observed that the differences between the highest value (933.50 gm/kg) and the lowest value (869.50 gm/kg) of water were 6.68% in respect of highest value (Table 2.3). The higher content of water in the samples of Local market of Sirjgonj and the samples of Sirajgonj town milk samples clearly indicated that some portion of water might have been added in their milk than other two places of Pabna district.

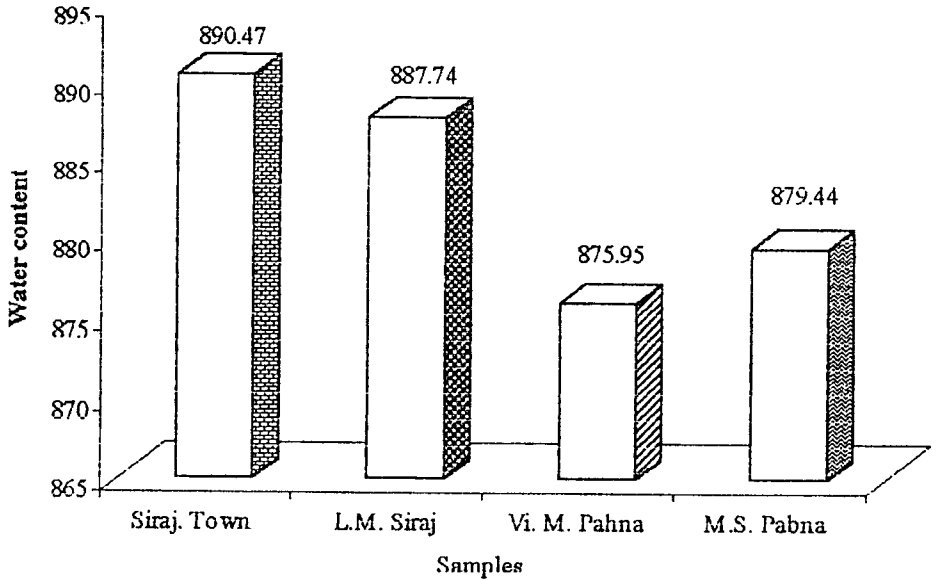

Fig. 2.6 Graph showing the average value of water content of milk in northern part of Bangladesh

3.2.3.3 Test for detecting adulteration

a) Percent of water added: The average values and standard deviations of percent of water added in milk collected from four places were 9.19±12.39, 11.53±2.31, 4.78±2.75 and 9.05±2.83 respectively (Table 2.2 and Fig. 2.7). Statistical analysis showed that the difference between the percent of water added of milk samples collected from four different places was found significant ($P<0.05$) (Appen. Table 19). It was also observed that the difference between the highest value (45.3) and the lowest value (0.00) of percent of water added were (100%) in respect of highest value (table 2.3). It was found that the average percent of water added of milk samples collected from Pabna district was lower than that of milk samples collected from two places of Sirajgonj district. Higher water percentage of milk samples indicating the adulteration of milk samples with water. The milk samples collected from Pabna and Sirajgonj district may not be adulterated with specific adulterants like (sugar and flour added and preservatives like Formalin and $NaHCO_3$) were tested in the Laboratory. But no adulterants were found out of 80 samples from the above sources. Statistical analysis showed that the differences between the percent of water added of milk samples collected from different four places was found significant ($P<0.01$).

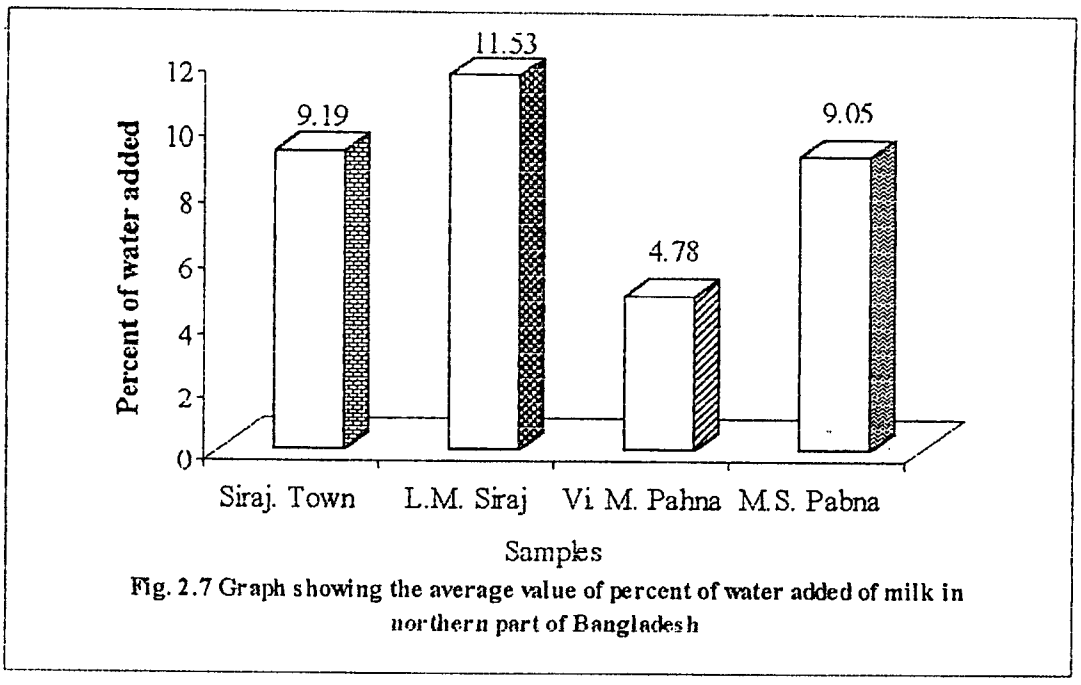

Fig. 2.7 Graph showing the average value of percent of water added of milk in northern part of Bangladesh

3.2.3.4 Microbiological Parameters

a) Total viable bacterial count: The average values of total viable count/ml. of milk samples collected from Sirajgonj town, Local market of Sirajgonj, Village market of Pabna and milk shed area (society) of Pabna were 36400 CFU/ml (log 4.56), 28800 CFU /ml (log 4.46), 29650 CFU/ml (log 4.47) and 31250 CFU /ml (log 4.49), respectively (Table 2.4 and Fig. 2.8). Statistically it was found that there were significant differences ($P<0.01$) (Appen. Table 20) within the total viable bacteria of milk samples collected from four different places of Sirajgonj and Pabna district. It was also observed that the highest value (45000 CFU/ml) and the lowest value (22000 CFU/ml) of total viable count /ml were 51.11% in respect of highest value (Table 5). It was found that the average value of total viable bacteria obtained from Sirajgonj town (36400 CFU/ml) was significantly higher and milk sample of Local market of Sirajgonj (28000 CFU/ml) was lower than the total viable bacteria of milk of other samples.

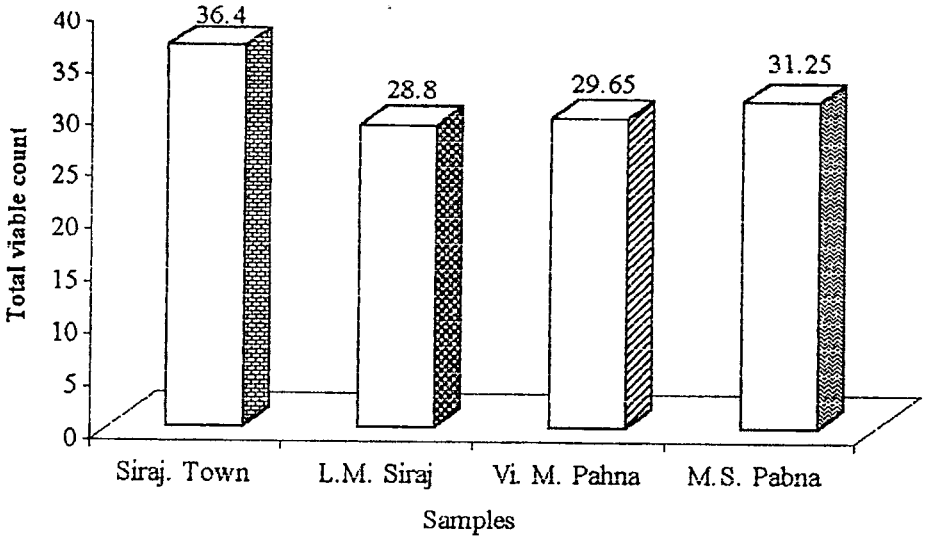

Fig. 2.8 Graph showing the average value of total viable count of milk in northern part of Bangladesh

b) Coliform Bacteria Count: The average values of coliform counts /ml of milk samples collected from four places were 94.0 CFU/ml (log 1.97), 89.5 (log 1.95), 89.0 (log1.95) and 83.5 (log 1.92) respectively (Table 2.4 and Fig. 2.9). Statistically it was found that there were non- significant differences within the coliform bacteria of milk samples collected among the four places of northen part of Bangladesh (Appen. Table 21). It was also observed that the highest value (150 CFU/ml) and the lowest value (60 CFU/ml) of coliform bacteria count/ml of milk were 60% in respect of highest value (Table 2.5). It was found that the coliform bacteria of Sirajgonj town milk samples was higher and the milk samples of milk shed area (Society) of Pabna was lower than the other two places. Coliform bacteria are one of the major indications of hygienic condition of milk. Usually good quality milk contains 10 coliform bacterial/ml of milk. But in this experiment coliform count of all milk samples were more than the normal value indicating that proper hygienic condition is not follower by the farmers.

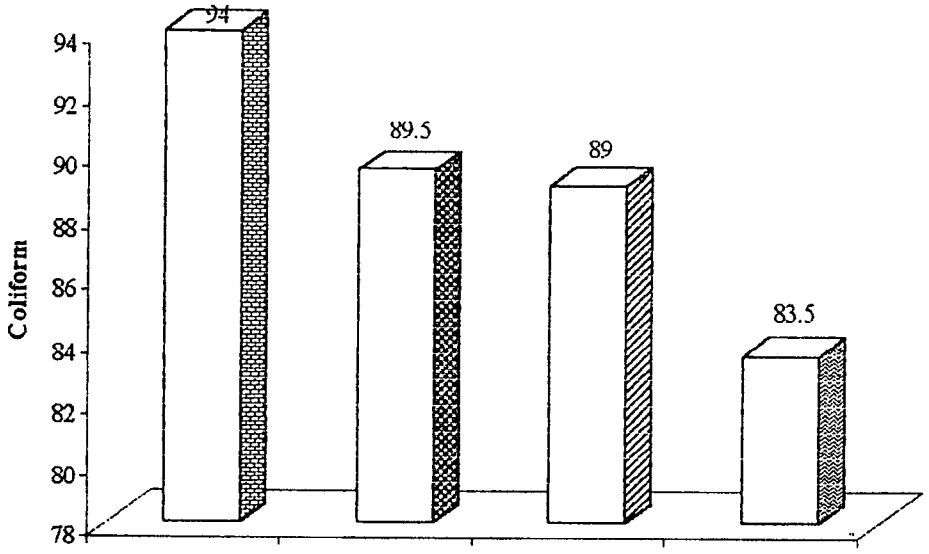

Fig. 2.9 Graph showing the average value of coliform count of milk in northern part of Bangladesh

Table 2.4 Average total viable count and coliform count in raw milk of four different places of Pabna and Sirajgnj district.

Sources of milk	No. of samples analysis	Total count/ml.		Coliform count/ml.		Level of significance	LSD value
		CFU/ml	log.	CFU/ ml	log.		
Sirajgonj town	20	36.400	4.56	94.0	1.97	TVC ** Coliform count =NS	TVC– 3038.68 Coliform count=NS 13.81
Local market of Sirajgonj	20	28.800	4.46	89.5	1.95		
Village market of Pabna	20	29.650	4.47	89.0	1.95		
Milk shed area (Society) of Pabna	20	31.250	4.49	83.5	1.92		

All counts are expressed in logarihms

**= Significant at 1% level

NS= Non significant

Table 2.5 Summary of the results of total viable bacteria and coliform bacteria per ml of 80 milk samples.

Parameter	Minimum Values	Maximum Values	Mean	andard Deviation	% difference
Total viable bacteria per Ml (x 1000)	22.000	45.000	31525.0	5374.5	51.11
Coliform bacteria per ml (x 10)	60	150	89	20.721	60.0

SUMMARY AND CONCLUSION

The experiment was conducted for a period of six months starting from May to October, 2001-2002. The parameters used to monitor the physical, chemical and microbiological qualities of milk samples were as follows.

1. Physical tests: colour, taste, flavour and specific gravity of milk samples
2. Chemical test: acidity, fat, solids-not-fat, total solids, water content and detecting percent of water added.
3. Microbiological tests: total viable bacteria count and coliform bacteria count.

From the organoleptic test there were no marked differences observed among the milk samples regarding colour, taste and flavour of milk collected from four different places of Sirajgonj and Pabna district. The colour, taste and flavour of milk samples were normal i.e. golden yollowish white, slightly sweet and pleasant aromatic. Only 3 samples (15.1%) were abnormal (flat) in taste. The colour, taste and flavour of milk varies depending on many factors. The mean and standard deviation of specific gravity of milk samples collected from Sirajgonj town, Local market of Sirajgonj, Village market of Pabna and milk shed area (Society) of Pabna were 1.029±0.00, 1.027 ±0.00, 1.029±0.00 and 1.027±0.00 respectively. The specific gravity of milk samples collected from the above places were within the normal range. From chemical analysis it was

observed that the average content of fat, solids not fat, total solids and water obtained from four different places were 31.50±4.97, 37.10±2.27, 43.25±3.10, 43.25±2.07; 78.05±11.32, 75.17±1.96, 80.90±2.37, 77.27±2.41; 109.56±15.23, 112.26±2.01, 124.15±2.62, 120.54±3.49; 890.47±15.22, 887.74±0.01, 875.95±2.61 and 879.44±3.47 gm/kg respectively. The percent of acidity and the percent of water added in the same order were 0.154±0.01, 0.148±0.00, 0.151+0.00, 0.152+0.01 and 9.19+12.39, 11.53+2.31, 4.78+2.75, 9.05+2.83 respectively. Statistical analysis showed that the different parameters of milk samples among the four different places varied significantly ($P<0.01$, $P<0.05$). The average values of total viable bacteria count/ml of milk samples collected from Sirajgonj town, Local market of Sirajgonj, Village market of Pabna and milk shed area (Society) of Pabna were 36400 CFU/ml (log 4.56), 28800 CFU/ml (log 4.46), 29650 (log CFU/ml (log 4.47) and 31250/CFU /ml (log 4.49) respectively (table 4). Statistically there were significant ($p<0.01$) differences within the total viable bacteria /ml of milk samples collected from the above-mentioned places of Pabna and Sirajgonj district. The average values of coliform counts /ml of milk samples collected from the above places were 94.0 CFU/ml (log 1.97), 89.5 CFU/ml (log 1.95), 89.0 (log 1.95) and 83.5 CFU/ml (log 1.92) respectively. Statistically it was found that the were non-significant differences within the coliform bacteria of milk samples collected from four places of northern part of Bangladesh. Coliform bacteria are one of the major indications of hygienic condition of milk. For the production of "better quality milk" in the rural condition better hygienic condition to be followed to reduce the incidence of bacteria in milk.

Experiment 3 Effect of sodium bicarbonate on the keeping quality of milk

INTRODUCTION

Milk is an ideal and almost complete food having all the nutrients essential for normal functioning of the body system. The nutritive value of milk depends on its freshness, cleanliness, purity and wholesomeness. For this reason, the time interval between milk collected from the small farmers to the consumers is primarily most important to ensure fresh, clean, pure and wholesome milk. There is every possibility of spoilage of milk during that time. So, it is very important to adopt some technique for increasing the shelf life of milk. The presence of different types of microorganisms or undesirable bacteria in milk may cause deterioration of flavour, colour, taste or physical appearance. At the same time spoilage takes place rapidly due to the formation of excess lactic acid from the break down of lactose by lactic acid producing bacteria. To make the milk safe for public health and also to increase its shelf life it is very important to preserve milk scientifically.

Due to lack of proper milk preservation facilities, a huge quantity of milk undergoes spoilage every year in our country. Milk can be preserved for a while for human consumption by using some chemical substances such as hydrogen peroxide (H_2O_2) (Hossain, 1989), sodium bicarbonate (Kukde, 1991), etc. and by regulating the temperature i.e. cooling, pasteurization and boiling. Cooling and pasteurization facilities are not available throughout the country. At the same time, heated boiled milk is not also popular in our country. Established dairy farms have modern facilities for milk preservation but small farmers or the poor farmers or vendors or goalas who live in rural areas have no such types of facilities for milk preservation. Most of the farmers or vendors are illiterate and they do not know how to preserve milk scientifically. It is urgently needed to develop low cost short time milk

preservation technology in order to reduce the spoilage of milk which usually occurs during transportation and keeping long time without applying any scientific technique before marketing.

So, in this experiment an attempt was made to preserve milk samples by sodium bicarbonate ($NaHCO_3$). It is well known that sodium bicarbonate is a cheap and available alkaline substance used mostly in the bakery for preparation of different types of cakes, breads and biscuits. So, it is expected that handling of this chemical will be very easy by the farmers and their will be no hazards effects on public health. Local goalas or vendors or farmers are using this chemical for milk preservation but scientifically its feasibility as milk preservative has not been carried out widely. Mahboob (1992) from a preliminary study found that sodium bicarbonate is useful for short time preservation of milk. Hence this experiment was conducted to monitor the usefulness of sodium bicarbonate ($NaHCO_3$) as milk preservative.

MATERIALS AND METHODS

3.3.2.1 Time and place of experiment

The experiment was conducted at Dairy Technology and Dairy Microbiology Laboratory of the Department of Dairy Science, Bangladesh Agricultural University, Mymensingh during the period of May to October, 2001.

3.3.2.2 Source of milk

Whole milk was collected from Bangladesh Agricultural University dairy farm. Suggestions were given to the milkers before milking the cows for maintaining all hygienic measures. Milk was poured from one pail to another after milking. To avoid the incorporation of air it was allowed to stand for a while and thereafter milk was taken to the Laboratory for experimental purpose.

3.3.2.3 Experimental procedure

The collected milk samples after thoroughly mixing was divided into four equal parts. Out of four parts, one was kept as whole milk (control) without $NaHCO_3$ and the other three parts were preserved with different levels of $NaHCO_3$.

The four treatment groups were :

(1) Milk sample without $NaHCO_3$.(control)

(2) Milk sample with 0.1% $NaHCO_3$.

(3) Milk sample with 0.15% $NaHCO_3$.

(4) Milk sample with 0.2% $NaHCO_3$.

The parameter used to monitor the physical, chemical and microbiological qualities of milk were determined initially just before adding $NaHCO_3$ and then after two hours interval upto 12 hours and thereafter every one hour interval until the milk samples were spoiled.

Tests

The following tests were performed with each milk sample:

(1) Physical test

a) Organoleptic tests

i) Colour

ii) Flavour

iii) Texture

b) Specific gravity

(c) Clot-on-boiling (COB) test

(d) Alcohol test.

(2) Chemical test

i) Acidity (%).

Organoleptic tests were performed with an expert pannel of judges. Specific gravity, clot-on-boiling and alcohol test was done as per method described by Aggarawala & Sharma (1961).

3.3.2.4 Statistical Analysis

Statistical analysis was done by using Randomized Complete Block Design (RCBD) as per steel and Torrie (1980). Analysis of variance was done to find the statistical difference (Significant or not) between the different treatments and in case of significant difference LSD value was calculated to make a comparison between treatment means.

RESULT AND DISCUSSION

In this experiment, an attempt was made to preserve milk samples with different levels of sodium bicarbonate ($NaHCO_3$). Results obtained from this study are presented below :

3.3.3.1 Initial quality of milk

The physical, chemical and microbiological qualities of milk were determined before adding $NaHCO_3$ with milk sample. Results obtained from initial analysis are presented in Table 3.1.

Table 3.2 Colour quality of control and different proportions of sodium bicarbonate treated milk samples during preservation period

Hour	Control	Treatments		
		0.1%	0.15%	0.2%
0-8th hour	Golden yellowish white	Golden yellowish white	Golden yellowish white	Golden yellowish white
10th hour	Golden yellowish white	Golden yellowish white	Golden yellowish white	Golden yellowish white
12th hour	Bleached	Golden yellowish white	Golden yellowish white	Golden yellowish white
13th hour	Bleached	Bleached	Golden yellowish white	Golden yellowish white
14th hour	Bleached	Bleached	Bleached	Golden yellowish white
15th hour	Bleached	Bleached	Bleached	Golden yellowish white
16th hour	Bleached	Bleached	Bleached	Golden yellowish white
17th hour	Bleached	Bleached	Bleached	Bleached

In this experiment, normal colour of milk samples indicated that no fat had been removed or fat percent in milk was not too low before starting the experiment. The colour of whole milk (control) and NaHCO3 treated milk samples are shown in table 3.2. From the Table 3.2, it is evident that for whole milk (control), 0.1, 0.15 and 0.2 percent $NaHCO_3$ treated milk samples, colour was normal upto 12,13,14 and 17 hours respectively and after which colour became bleached. Colour deterioration was very rapid in whole milk (control) followed by 0.1, 0.15 and 0.2 percent $NaHCO_3$ treated milk samples. This indicates that $NaHCO_3$ could be used as a short term milk preservative under rural areas where scientific cooling or pesteurization facilities are not available.

ii) **Flavour:** The flavour of all milk samples before starting the experiment was normal (100%). All samples showed pleasing aromatic flavour. It has been

shown that the pleasing aromatic flavour of milk may be correlated with high lactose and a relatively low chloride content. A low lactose and a high chloride content probably would mean a milk with salty flavour (Eckles *et al.* 1951). Biswas (1997) found that flavour of all milk samples collected from BAU dairy Farm was normal.

Table 3.3 Flavour quality of control and different proportions of sodium bicarbonate treated milk samples during preservation period

Hour	Treatment			
	Control	0.1%	0.15%	0.2%
0-8th hour	Pleasing	Pleasing	Pleasing	Pleasing
10th hour	Pleasing	Pleasing	Pleasing	Pleasing
12th hour	Pleasing	Pleasing	Pleasing	Pleasing
13th hour	Slightly sour	Pleasing	Pleasing	Pleasing
14th hour	Slightly sour	Slightly sour	Pleasing	Pleasing
15th hour	Sour	Sour	Slightly sour	Pleasing
16th hour	Sour	Sour	Sour	Pleasing
17th hour	Bitter	Sour	Sour	Slightly sour

In this experiment, flavour of whole milk (control), 0.1, 0.15, and 0.2 percent $NaHCO_3$ treated milk samples were acceptable upto 13,14,15 and above 16 hours respectively in table 3.3. After that time, the flavour was becoming unacceptable. This result showed that $NaHCO_3$ is effective for controlling the flavour of milk. This was due to the fact that in fresh milk lactic acid produced from the fermentation of lactose was not neutralized. But in $NaHCO_3$ treatment milk samples

lactic acid produced was neutralized by $NaHCO_3$ and hence the keeping quality of milk was increased.

iii) **Texture:** All milk samples before starting experiment was normal in Texture. The texture of all milk samples shown in table 3.4. Texture of normal milk is designated as free flowing liquid. Its viscosity is higher than water. Total solids content of milk are dissolved in water of milk. Some solids exits in true solution phase, some are at colloidal state and some other portions as coarse dispersion phase Texture of milk changes if some portion of fat is removed or water is added for adulteration purpose. In such case, milk becomes less viscous. Acidity development can also changes the texture of milk.

Table 3.4 Texture quality of control and different proportions of sodium bicarbonate treated milk samples during preservation period

Hour	Treatments			
	Control	0.1%	0.15%	0.2%
0-8th hour	Free flowing	Free flowing	Free flowing	Free flowing
10th hour	Free flowing	Free flowing	Free flowing	Free flowing
12th hour	Slightly clotted	Free flowing	Free flowing	Free flowing
13th hour	Slightly clotted	Slightly clotted	Slightly clotted	Free flowing
14th hour	Clotted	Slightly clotted	Slightly clotted	Free flowing
15th hour	Clotted	Clotted	Clotted	Free flowing
16th hour	Clotted	Clotted	Clotted	Free flowing
17th hour	Curd	Curd	Curd	Slightly clotted

So the present results indicated that milk collected from BAU Dairy Farm was fresh and no fat had been removed from the milk. The normal texture of milk is stated as "free flowing liquid". From this table it is evident that the texture of whole milk (control), 0.1, 0.15 and 0.2 percent $NaHCO_3$ treated milk samples were normal up to 12.13, 14 and above 16 hours of study respectively. Thereafter samples become clotted. The whole milk (control) sample clotted earlier than $NaHCO_3$ treated milk samples. The clotting time depends upon the percent of $NaHCO_3$ used for preserving milk. Texture deterioration was rapid in fresh milk due to lactic acid production than treated milk with $NaHCO_3$.

iv) **Specific gravity:** Average specific gravity of milk samples was 1.030±0.0007. The specific gravity was within the normal range of specific gravity of milk. Generally the specific gravity of fresh milk are within the range of 1.027 to 1.035 having an average value of 1.032 (Eckles, 1951) Adulteration of milk by adding water decreased its specific gravity. In our experiment, the average specific gravity of milk samples was within the normal range but slightly below the average specific gravity of milk (1.032). This might be due to high fat and slightly low SNF content of milk. Eckes *et al.* (1951) stated that as milk fat is the lightest constituents of milk, the more that is present, lower the specific gravity will be and in a like manner, the greater the percentage of SNF, the heavier the milk will be. Similar type of specific gravity was obtained by Biswas (1997) for BAU dairy farm milk.

v) **Clot-on-boiling test:** The results of acidity tests were confirmed by clot–on–boiling (COB) test. The test showed negative results indicated that there was no developed acidity and the quality of the milk samples was good. The results of COB test are shown in (Table 3.5 and Fig. 3.1). The COB test was positive at

13.05± 0.229, 14.08± 0.07, 15.30± 0.06 and 17.01± 0.3 hours for whole milk (fresh), 0.1, 0.15 and 0.2 percent $NaHCO_3$ treated milk samples respectively. From this result it is clear that whole milk (control) sample clotted earlier than that of $NaHCO_3$ treated milk samples. This was due to more acid production in fresh milk samples. On the other hand, $NaHCO_3$ neutralized the acids produced by lactic acid producing bacteria from the break down lactose. Clot-on-boiling test confirms the results of acidity test. This test also indicates that $NaHCO_3$ could be used as milk preservative under village or rural areas of Bangladesh. The result of this study agrees with the results of Hussain (1989), El- safety *et al.* (1978) and Barabas (1995).

Table 3.5 Average positive (COB) time of milk treated under various treatments

Hour after treatment	Treatments			
	Control	0.1%	0.15%	0.2%
0-12th hour	-	-	-	-
13th hour	+	-	-	-
14th hour	+	+	-	-
15th hour	+	+	-	-
16th hour	+	+	+	-
17th hour	+	+	+	+
Average COB positive time	13.05 ± 0.29	14.08 ± .07	15.30± 0.06	17.01 ± 0.3

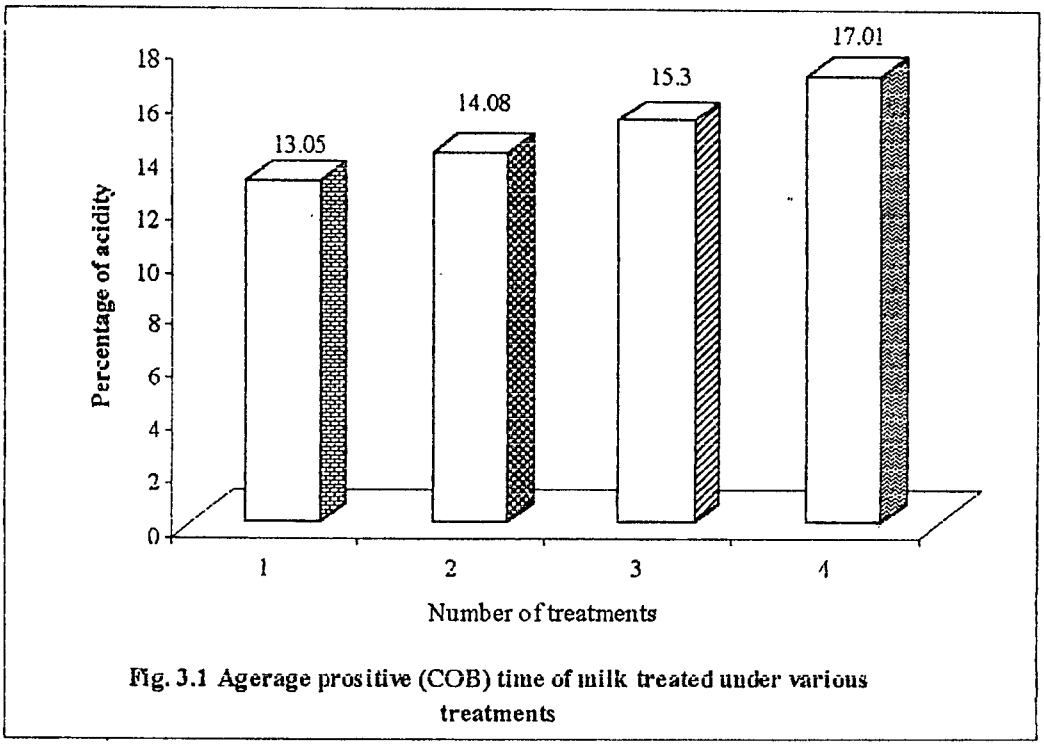

Fig. 3.1 Agerage prositive (COB) time of milk treated under various treatments

vi) **Alcohol test** :Initially alcohol test was negative for all type of samples but the alcohol test was positive at 12.30+ 0.06, 13.45 + 0.08, 15.01+ 0.28 and 16.39+ 0.08 hours for fresh milk, 0.1, 0.15 and 0.2 percent $NaHCO_3$ treated milk samples respectively (Table 3.6 and Fig. 3.2). The alcohol test confirmed the results of COB test. It is clear that fresh milk samples clotted earlier than that of $NaHCO_3$ treated milk samples. Table 3.6 Average positive Alcohol time of milk treated under various treatments.

Table 3.6 Average psotove Alcohol time of milk treated under various treatments

Hour after treatment	Treatments			
	Control	0.1%	0.15%	0.2%
0-12th hour	-	-	-	-
13th hour	+	-	-	-
14th hour	+	+	-	-
15th hour	+	+	+	-
16th hour	+	+	+	-
17th hour	+	+	+	+
Average alcohol positivc timc	12.30 ± 0.06	13.45 ± 0.06	15.01± 0.28	16.39± 0.08

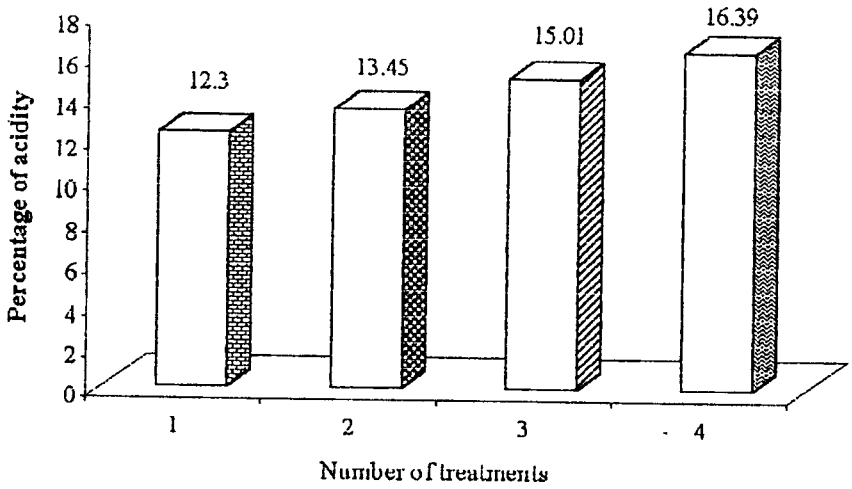

Fig. 3.2 Agerage prositive alcohol time of milk treated under various condition

3.3.3.3 Chemical parameters

Acidity test: Results of acidity test are shown in table 3.1. Mean initial acidity of experimental samples was 0.15± 0.002. Generally acidity of normal milk samples are within the range of 0.10 to 0.20 (Eckles, *et al.* 1951). Similar types of acidity (0.13%) was reported by Biswas (1997) for BAU dairy farm milk. Acidity test of milk is a good indicator of milk quality. Fresh milk shows an acidity of about 0.15% which is due to the presence of citrate, phosphate, carbon-dioxide and milk casein. If the milk samples is kept for several hours without pasteurization or cooling or any kind of heat treatment then its lactose undergoes fermentation and produces lactic acid in milk. This additional acidity is known as developed acidity and is responsible for quick spoilage of milk. Acidity results of our samples indicated that there was no developed acidity in milk samples and its quality was good. The results of average acidity of control and different proportions of sodium bicarbonate treated milk samples during the preservation period are presented in table 3.7. Acidity of experimental samples were measured on every two hours interval upto 12 hours and thereafter every one hour interval starting from zero (0)

to 17 hours (Table 3.7 and Fig. 3.3). Overall mean acidity changes with time (time and treatment effect) for 17 hours of study of control and different proportions of $NaHCO_3$ treated milk samples are shown in Table 3.8.

Mean acidity of fresh milk (control), 0.1, 0.15 and 0.2 percent $NaHCO_3$ treated milk samples were 0.188± 0.05, 0.176 ±0.04, 0.168±0.03 and 0.159±0.02 respectively (Table 3.9).

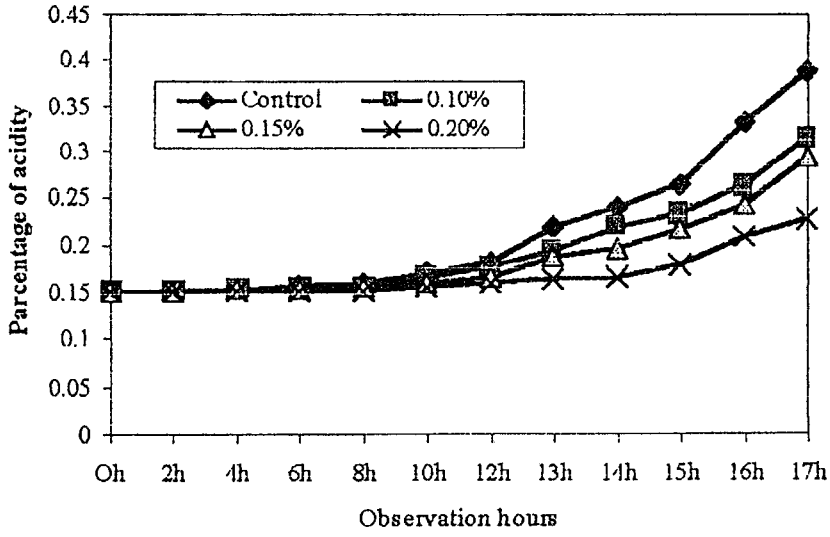

Fig. 3.3 Average acidity of control and different proportions of sodium bicarbonate treated milk samples during preservation period

Statistical analysis showed that there were significant differences ($P<0.01$) within the mean acidity of milk samples of control and different proportions of $NaHCO_3$ treated milk samples (Appen. Table 22). Mean acidity of milk preserved with 0.2 percent $NaHCO_3$ was significantly ($P<0.01$) lower than that of control milk sample. Acidity production had an important relationship with time. Acid production increased significantly ($P<0.01$) with increase in time. Table 8 showed that acid production was low at the beginning but was very high at the end of 17 hours of study. Interaction effect of treatment and time of milk also significant ($P<0.01$). Bogdanova *et al.* (1976) found that acidity increased with storage time. Duncan *et al.* (1991) also reported that average daily acidity increased with storage time.

Table 3.7 Average acidity of control and different proportions of sodium bicarbonate treated milk samples during preservation period

Time (hour)	Treatments			
	Control	0.1%	0.15%	0.2%
'0' hour	0.150	0.150	0.150	0.150
2nd hour	0.150	0.150	0.150	0.150
4th hour	0.152	0.152	0.152	0.150
6th hour	0.156	0.154	0.152	0.150
8th hour	0.159	0.154	0.154	0.150
10th hour	0.17	0.165	0.158	0.154
12th hour	0.181	0.175	0.165	0.158
13th hour	0.218	0.192	0.186	0.163
14th hour	0.241	0.218	0.194	0.165
15th hour	0.264	0.234	0.216	0.178
16th hour	0.332	0.264	0.243	0.207
17th hour	0.387	0.315	0.294	0.228
LSD Value	0.017			

Table 3.8 Average acidity changes with time under various treatments

Treatment	% acidity	Ranked
T_0	0.150 ± 0	E
T_2	0.150 ± 0	E
T_4	0.152 ± 0	E
T_6	0.153 ± 0.001	DE
T_8	0.154 ± 0.003	DE
T_{10}	0.161 ± 0.008	D
T_{12}	0.170 ± 0.01	C
T_{14}	0.204 ± 0.03	B
T_{16}	0.262 ± 0.05	A
Level of significance	**	
LSD value	0.008	

Table 3.9 Effect of treatment on acidity of control and different proportion of $NaHCO_3$ treatment milk sample

Treatment	% acidity	Ranked
Tr_1	0.188 ± 0.05	A
Tr_2	0.176 ± 0.04	B
Tr_3	0.168 ± 0.03	C
Tr_4	0.159 ± 0.02	D
Level of significance	**	
LSD value	0.0055	

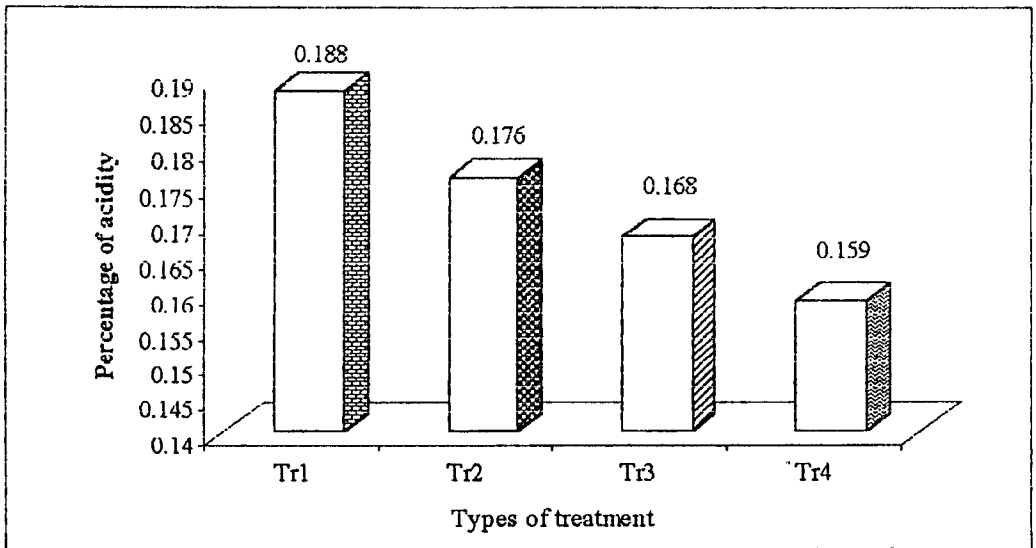

Fig. 3.4 Average acidity of different treatments during experimental period

SUMMARY AND CONCLUSION

The present experiment was carried out to know the effect of sodium bicarbonate on the keeping quality of raw milk. For this purpose, milk samples were collected from Bangladesh Agricultural University Dairy Farm. Initial quality of the collected milk samples were evaluated at Dairy Technology Laboratory of the Department of Dairy Science through some physical and chemical tests. Thereafter collected samples were preserved at room temperature (32-34°C) with 0.10, 0.150 and 0.20 percent sodium bicarbonate. One group was kept without sodium bicarbonate and was considered as control group. The quality of milk samples were measured at every two hours interval upto 12 hours and thereafter every one hour interval until spoilage. Initially, colour, flavour and texture of all milk samples were normal (100%), but with progressive storage time colour, flavour and texture of all samples deteriorated gradually. The deterioration was more rapid for control samples than that of the sodium bicarbonate treated samples. Acidity percent of all samples increased gradually during storage period and the differences in acidity of milk samples in different treatments were significant ($p<0.01$). The result of acidity test was supported by COB test. Control samples spoiled after 12 hours but that of 0.10, 0.15 and 0.20 percent sodium bicarbonate treated samples spoiled after 13,14 and 16 hours respectively. It may be concluded that $NaHCO_3$ is the effective chemical for neutralizing the acids produced by acid producing bacteria and can be used as a short term preservation of milk under rural condition of Bangladesh where scientific cooling or pasteurization facilities are not available.

Experiment 4 Effect of container on the storage life of milk

INTRODUCTION

Milk is an almost ideal food. It is essential for healthy development and growth of young and is an ideal drink for all ages. It is such a food which contains a high percentage of water in which other components like fat, protein, lactose, vitamins and minerals are dissolved or suspended. It has high nutritive value. It supplies body-building proteins, bone-forming minerals, health-giving vitamins and furnishes energy-giving lactose and milk fat. Besides supplying certain essential fatty acids, it contains the above nutrients in an easily digestible and assimilable form. All these properties make milk an important food for pregnant mothers, growing children, adolescents, adults, invalids, convalescents and patients alike. Although milk is an ideal food for human being but if can be spoiled very quickly and its nutritive value may be deteriorated if it is not stored properly Milk can undergoes spoilage due to the action of microorganisms. Lactose is broken down to glucose and galactose by microbial enzyme lactase and finally lactic acid is produced from glucose. If the concentration of lactic acid increases, then the quality of milk is deteriorated. At the same time quality of milk may also be deteriorated due t the action of various metatic substances on milk. Sometimes small amount of metal can dissolve from container and the metallic salts thus formed may give rise to a metallic taste in milk. Some metals may act as catalyst and thus hastens spoilage of milk (Sukumar DE2000).

In our country villagers or milk businessmen use different types of containers to store milk. They don't have any idea about the effects of containers on storage life of milk. Islam et al (1982) from an experiment observed that containers had some effects on storage life of ghee. From a preliminary study Rahman *et al.* (1992) found that aliminium container was suitable for storage of milk. No systematic or elaborate work has yet been done in our country to monitor the effect of container on keeping quality of milk. So, in this experiment an attempt was made to observe the effects of containers on storage life of milk both in summer and winter season of the year.

MATERIALS AND METHODS

This experiment was conducted to evaluate the effect of containers on keeping quality of milk.

3.4.2.1Time and place of experiment

The experiment was conducted at the Dairy Technology and Dairy Microbiology Laboratory of Department of Dairy Science, Bangladesh Agricultural University, Mymensingh during the period of May to October, 2001(summer) and November to February, 2002(winter).

3.4.2.2 Experimental procedure

The experiment was conducted in two phases. The first phase of the study was carried out in summer season, (May to October, 2001) and the second phase was carried out in winter season (November to February, 2002). In each phase six different types of containers such as stainless steel, aluminium, glass, plastic, tin and earthen container were used for experimental purpose. All containers were uniform in size. In each phase milk samples were collected from Bangladesh Agricultural University Dairy farm. The collected sample were initially analysed in the laboratory to monitor their quality and thereafter were divided into six equal portions. These six milk samples were kept randomly in six different types of containers. About one litter of milk samples were put in each containers and were stored at room temperature until spoilage. The trials were given in each phase and in each trial three replications were for each container. The room temperature throughout the study period ranged from 18-22 ^{0}c at winter and 31.1 to 31.7°c at summer season. The layout of the experiment is shown in table 4.1.

Table 4.1 Experimental layout

Type of milk	Container used	Replication
Milk at summer Season at room Temperature	Stainless steel container	3
	Aluminum container	3
	Glass container	3
	Plastic container	3
	Tin container	3
	Earthen container	3
Milk at Winter season at room temperature	Stainless steel container	3
	Aluminum container	3
	Glass container	3
	Plastic container	3
	Tin container	3
	Earthen container	3

The parameters used to determine the quality of milk during storage period in both phase were physical (colour, flavour, texture and Specific gravity), chemical (acidity, alcohol and COB test) and microbiological (total viable count and coliform count). Colour, flavour and texture characteristics were done organoleptically and specific gravity, acidity, alcohol and COB tests were done as per Aggarwala and Sharma (1961). Microbiological parameters were determined as per American Public Health Association (APHA, 1972).

3.4.2.3 Statistical Analysis

The experiment was conducted in Randomized Complete Block Design (RCBD) with 2 factors; factor 1 was time and factor 2 was container. Analysis of variance (ANOVA) test was done to findout the difference between treatments. In case of significant difference, Duncan's Multiple range Test (DMRT) was carried out to find out significant difference between treatment means.

RESULTS AND DISCUSSION

The keeping qualities of milk were monitored during the storage period by determining the percentage of acidity as well as clot-on-boiling and alcohol tests in both phases of the experiment.

Phase 1 : Effect of containers on the keeping quality of milk in summer season

3.4.3.1 Initial quality of milk

Initial quality of milk used in first phase of the study are shown in table 4.2. From the table it is evident that average fat, protein, lactose, ash, solids -not –fat and total solids content of milk samples were within normal range. Similarly specific gravity, colour, flavour and texture of milk samples were normal. Average specific gravity of milk samples was 1.0294±0.0005. There were no marked differences observed among the raw milk samples in regarding with the colour, flavour, taste and texture of milk from BAU dairy farm. Acidity, alcohol and COB tests confirmed that quality of milk used in the study was fresh and there was no developed acidity in any milk samples. Total viable bacteria and coliform count was also within normal limit. So, it is clear that milk used in this study to observe the effects of containers on their storage life was normal. During storage period the quality of milk samples were evaluated with the help of organoleptic (colour, flavour, texture and specific gravity), chemical (acidity, alcohol and COB test) and microbiological studies (Total viable bacterial count).

Table 4.2 Observation of the quality of milk at the beginning of the experiment at summer season.

Sl. No.	Parameters	Initial quality of milk before starting the experiment
1	Fat (g/kg)	47.8±2.28
2	SNF (g/kg)	83.6±0.97
3	TS (g/kg)	130.85±1.57
4	Water (g/kg)	869.14±1.6
5	Protein (g/kg)	35.6±0.55
6	Lactose (g/kg)	40.66±0.72
7	Ash (g/kg)	6.8±0.07
8	Specific gravity	1.0294±0.0005
9	Colour (% of normal condition)	Normal (100%) Yellowish white
10	Flavour (% of normal condition)	Normal (100%)
11	Texture (% of normal condition)	Normal (100%)
12	Alcohol test	(-Ve)
13	Clot –on –Boiling (COB)	(-Ve)
14	Acidity (%)	0.146±0
15	Temperature	31.1-31.7^{0}C
16	Total viable count	45000 CFU/ml
17	Coliform count	130 CFU/ml.

3.4.3.2 Physical parameters

(a) Organoleptic evaluation: At the stating of the experiment colour, flavour, and texture of all milk samples were normal (100%). It was found that with progressive storage time flavour and texture of all samples deteriorated and the deterioration was more rapid in tin and earthen containers followed by plastic, glass, aluminium and stainless steel containers. Changes in colour was very negligeable. In tin and earthen containers flavour became slightly acidic and texture became slightly thick at 12 hours and the samples became unacceptable. On the other hand in plastic, glass, and aluminium containers flavour and texture became unacceptable after 14 hours. Stainless steel container showed better in terms of keeping quality where colour and texture of milk samples became unacceptable after 15hours. Colour of milk samples in all types of containers did not show any appreciable change in any container during storage period. The result of organoleptic study in agrees with the findings of Mahboob (1992) who observed flavour deterioration was more in earthen container and less in aluminium container. Mahboob (1992) did not use stainless steel container. In our experiment it was found that flavour deterioration was less in stainless steel container and next was aluminium container. Tin containers gave similar performance to earthen container. Olson (1956) stated feed and weedy flavours develop in milk if the cow consumes onions, french weeds, bitter grass, bitter weeds, green rye etc. just before milking. Foley et al (1972) mentioned that cowy flavour is found in milk from cows suffering from ketosis. A bany flavour occurs in the milk of the cows housed in poorly ventilated sheds.

(b) Clot-on-boiling (COB) test: The results of acidity tests were confirmed by clot-on-boiling test. The test showed negative results in all the containers during the initial hour of the experiment indicated that there was no developed acidity and the quality of the milk samples was good. The results of COB test are shown in (Table 4.3 and Fig. 4.1). The COB test was positive at 15.30 ± 0.15, 14.30±0.15, 14.30±0.15, 14.0±0.15, 12.0±0.15 and 12.0±0.15 hours in stainless steel,

aluminium, glass, plastic, tin and earthen containers respectively. From this result it is clear that the milk samples in all containers clotted earlier than that of stainless steel container. This result is in agreement with Mahboob *et al.* (1992)

Table 4.3 Result of clot-on-boiling test (COB) in different containers with storage time in summer season

Period of storage (hours)	Stainless steel container	Aluminum container	Glass container	Plastic container	Tin container	Earthen container
0-8th hour	-	-	-	-	-	-
10th hour	-	-	-	-	-	-
12th hour	-	-	-	-	+	+
13th hour	-	-	-	-	+	+
14th hour	-	+	+	+	+	+
15th hour	+	+	+	+	+	+
16th hour	+	+	+	+	+	+
Average (COB) positive time	15.3± 0.15	14.3± 0.15	14.3± 0.15	14.0± 0.15	12.0± 0.15	12.0± 0.15

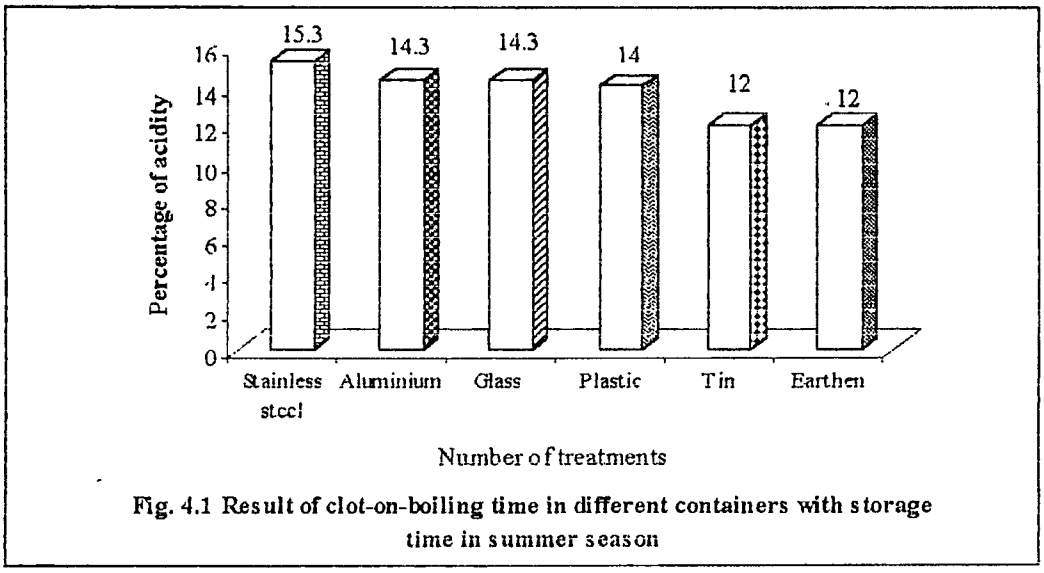

Fig. 4.1 Result of clot-on-boiling time in different containers with storage time in summer season

(c) Alcohol test: The alcohol test was positive at 15.03±0.05, 14.03±0.05, 14.08±0.07, 13.28±0.02, 11.30±0.05 and 11.32±0.02 hours in stainless steel, aluminium, glass, plastic, tin and earthen containers respectively. The alcohol test confirmed the result of acidity test. The only difference was that alcohol test gave positive result slightly carlier than COB test.

Table 4.4 Result of Alcohol test in different containers with storage time in summer season

Period of storage (hours)	Stainless steel container	Aluminium container	Glass container	Plastic container	Tin container	Earthen container
0-8th hour	-	-	-	-	-	-
10th hour	-	-	-	-	-	-
12th hour	-	-	-	-	+	+
13th hour	-	-	-	-	+	+
14th hour	-	+	+	+	+	+
15th hour	+	+	+	+	+	+
16th hour	+	+	+	+	+	+
Average alcohol positive time	15.03 ± 0.05	14.03 ± 0.05	14.08 ± 0.07	13.28 ± 0.02	11.30 ± 0.05	11.32 ± 0.02

3.4.3.3 Chemical parameters

(a) Acidity test : The result of average acidity percentage in all the containers during the study period are shown in table 4.5. It was found that initialy acidity of milk samples of all containers was normal (Table 4.2 and Fig. 4.2). The acidity of milk increased gradually in all types container with storage time. This was in

agreement with Duncan *et al.* (1991). Table 4.5 will give the idea about the changes of acidity in milk in different types of containers.

Table 4.5 Average acidity of milk in different containers changes with storage time during summer season

Period of storage (hours)	Stainless steel container	Aluminium container	Glass container	Plastic container	Tin container	Earthen container
0-0th hour	0.146	0.146	0.146	0.146	0.146	0.146
2nd hour	0.146	0.146	0.151	0.151	0.156	0.151
4th hour	0.151	0.151	0.156	0.156	0.160	0.165
6th hour	0.153	0.153	0.160	0.160	0.166	0.176
8th hour	0.160	0.160	0.165	0.166	0.181	0.186
10th hour	0.168	0.173	0.176	0.190	0.194	0.191
12th hour	0.175	0.180	0.186	0.195	0.204	0.204
13th hour	0.185	0.195	0.193	0.204	0.220	0.225
14th hour	0.201	0.210	0.210	0.214	0.240	0.242
15th hour	0.210	0.218	0.220	0.230	0.270	0.265
16th hour	0.225	0.230	0.250	0.261	0.304	0.304
x±SD	0.169±0.03	0.172±0.03	0.178±0.03	0.182±0.04	0.194±0.05	0.196±0.05

From the table it is clear that during 16 hours storage time average acidity in stainless steel, aluminium, glass, plastic, tin and earthen container were 0.225, 0.230, 0.250, 0.261b, 0.304, 0.304 and the percentage in acidity was 0.169±0.03, 0.172±0.03, 0.178±0.03, 0.182±0.04, 0.0194±0.05, 0.196±0.04 respectively in (Table 4.6). The overall mean acidity changes with time (time and treatment effect) for 16 hours of study period in different containers are shown in table 4.7. The differences in acidity content of milk samples in different container were significant ($P<0.01$) (Appen. Table 23). The rate of increase in acidity was higher in earthen container and lowest in stainless steel container. Acidity level was acceptable upto 15 hours in stainless steel container, 14 hours in aluminium, glass and plastic

containers, 12 hours in tin and earthen container. The result of acidity test was confirmed with the help of COB and alcohol test in this experiment. The room temperature ranged from 31.1-31.7^{0}C during the storage period was recorded.

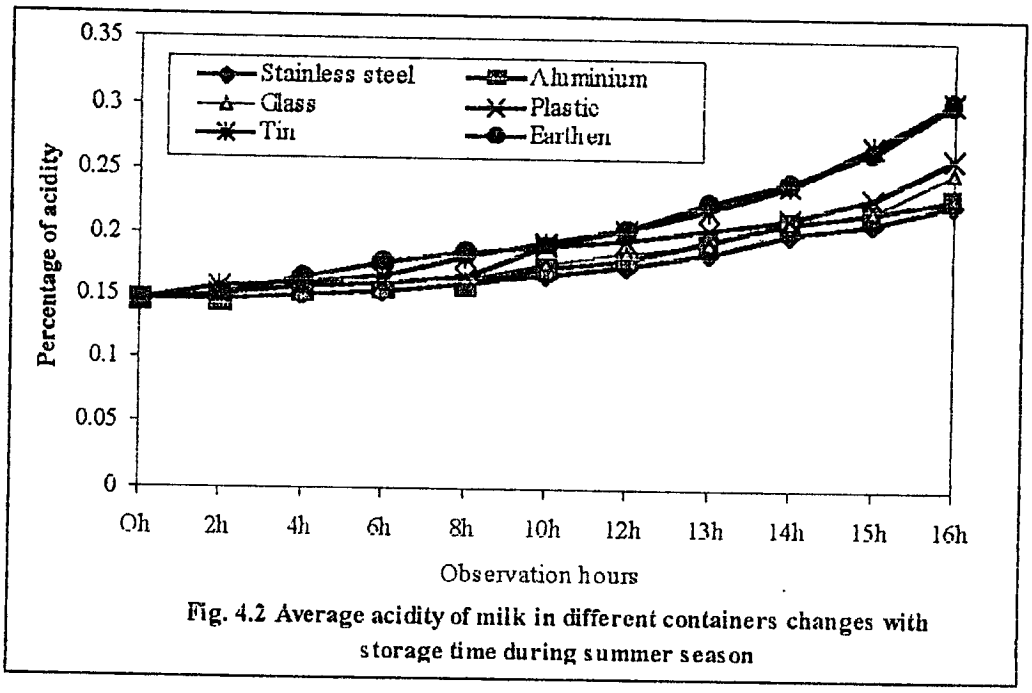

Fig. 4.2 Average acidity of milk in different containers changes with storage time during summer season

Table 4.6 Average acidity (Treatment effect) of milk in different containers in summer season

Treatment	% acidity
Tr_1	0.169 ± 0.03
Tr_2	0.172 ± 0.03
Tr_3	0.178 ± 0.03
Tr_4	0.182 ± 0.04
Tr_5	0.194 ± 0.05
Tr_6	0.196 ± 0.05
Level of significance	**
LSD value	0.0016

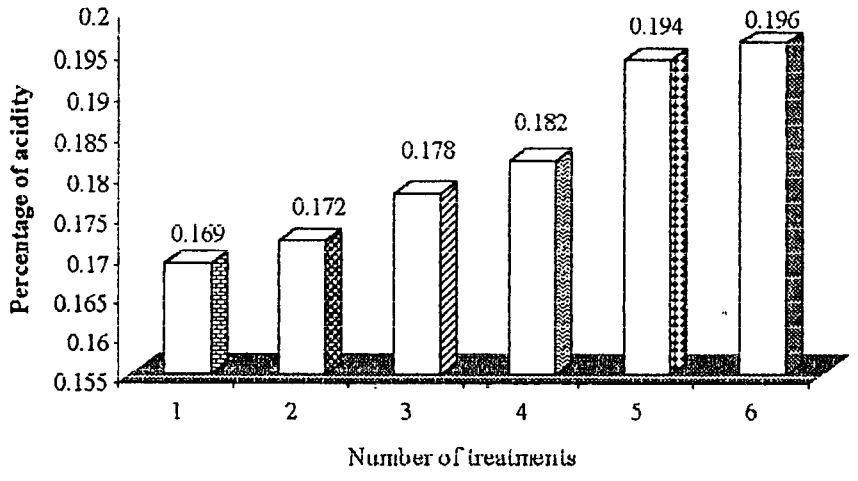

Fig. 4.3 Average acidity of different treatments during sixteen hours of study

Table 4.7 Average acidity changes with storage time during summer season

Time (Hours)	% Acidity changes	Ranked
T_0	0.146±00.00	I
T_2	0.151±0.003	H
T_4	0.157±0.005	G
T_6	0.162±0.008	F
T_8	0.170±0.01	E
T_{10}	0.184±0.01	D
T_{12}	0.195±0.02	C
T_{14}	0.216±0.01	B
T_{16}	0.267±0.04	A
Level of sig.	**	
LSD Value	0.002	

3.4.3.4 Microbiological parameters

Total viable and coliform bacteria count was done at the begining of the experiment and the number was same for milk preserved in each container (45000 CFU/ml and 130 CFU/ml). Thereafter total bacterial count was again done for the milk of each container when COB test gave positive result. So, during that time total bacterial count were 15000 CFU/ml, 200000 CFU/ml, 225000 CFU/ml, 240000 CFU/ml, 250000 CFU/ml and 255000 CFU/ml for the milk of stainless steel, aluminium,

glass, plastic, tin and earthen containers respectively. The result of present study agrees with the findings of Azad (1998), Patel *et al.* (1993).

Second phase: The second was done in winter season. Similar type of container and similar tests and procure which were used in first phase was also done in the second phase. The results obtained in the second phase are summarized below.

3.4.3.4.1 Initial quality of milk.

Initial quality of milk was also evaluated in the second phase of the study and the result obtained are presented in table 4.8. From the result it is evident that high quality milk was used for experimental purpose.

Table 4.8 Observation of the quality of milk at the beginning of the experiment at winter season.

Sl. No.	Parameters	Initial quality of milk before starting the experiment
1	Fat (g/kg)	48.0±2.3
2	SNF (g/kg)	84.6±0.98
3	TS (g/kg)	132.6±1.6
4	Water (g/kg)	867.4±1.7
5	Protein (g/kg)	36.0±0.6
6	Lactose (g/kg)	41.5+0.72
7	Ash (g/kg)	7.0±0.07
8	Specific gravity	1.030±0.0005
9	Colour (% of normal condition)	Normal (100%) Yellowish white
10	Flavour (% of normal condition)	Normal (100%)
11	Texture (% of normal condition)	Normal (100%)
12	Alcohol test	(-Ve)
13	Clot –on –Boiling (COB)	(-Ve)
14	Acidity (%)	0.145±0
15	Temperature	31.1-31.7^{0}C
16	Total viable count	41000 CFU/ml
17	Coliform count	110 CFU/ml.

3.4.3.4.2 Evaluation of milk during storage

Colour, flavour and texture quality of milk was evaluated organoleptically during the storage period. The result of organoleptic evaluation showed that the effect of containers on keeping quality of milk followed the similar trend which was obtained in the first phase of the study. In this case time difference was main findings. Flavour and texture of milk was normal upto 32 hours in stainless steel, aluminium, glass, plastic and earthen container. After that flavour became acidic and texture of milk became slightly thick. This was due to development of acidity during storage time. The colour of milk did not show any remarkable change during storage period. The result agrees with the findings of Duncan *et al.* (1992).

3.4.3.4.3 Acidity, alcohol and COB test

Results of the above tests are shown in table 4.9 and 4.10. Acidity of all samples at the start of the experiment was normal (0.145%). With progressive storage time acidity increased in all samples. The similar trend of result was seen in different containers in winter season also. Table 4.11 with give the idea about the changes of acidity in different types of containers. From the table the changes of acidity in stainless steel, aluminium, glass, plastic, tin and earthen containers were 0.210, 0.214, 0.217, 0.219, 0.228 and 0.228 respectively and the percentage of average was 0.156±0.02, 0.158±0.02, 0.159±0.02, 0.159±0.02, 0.162±0.02 and 0.162±0.02. It was also observed that when the room temperature was nearly 18^0C from 12^{th} hours to 24^{th} hour of treatment i.e. 7.0 pm to 7 a.m. the average acidity percentage in all containers remain unchanged due to lowering of temperature. Then the acidity of milk increased gradually with storage time (Table 4.11 and Fig. 4.5). The changes of acidity was higher in earthen container and lowest in stainless steel container. In winter season, milk was good upto 30 hours in all the containers. After 30 hours of storage the milk sample become spoilage in earthen and tin containers. The overall mean acidity changes with time (time and treatment effect) for 34 hours of study

period are shown in table 4.12. Alcohol and COB tests also showed same trend of result. However, alcohol test gave positive result slightly earlier than COB test. The result of above tests agrees with the finding of Mahbood (1992). The room temperature ranged from 18-22^{0}C was also recorded during the storage period. The COB and alcohol test confirmed the acidity test showed negative result in all containers upto 30 hours. Statistical analysis showed that there was significant difference ($P<0.01$) between containers in terms of keeping quality of milk. Ghafoor *et al.* (1985) indicated that raw milk could be preserved upto 12 hours without any preservative when the room temperature is about 29^{0}C).

Table 4.9 Result of clot-on-boiling test (COB) in different containers with storage time in winter season

Period of storage (hours)	Stainless steel container	Aluminium container	Glass container	Plastic container	Tin container	Earthen container
0-30th	-	-	-	-	-	-
31 th hour	-	-	-	-	-	-
32 th hour	-	-	+	+	+	+
33 th hour	-	+	+	+	+	+
34th hour	+	+	+	+	+	+
Average (COB) Positive time	34.03 ± 0.05	33.10 ± 0.08	32.28 ± 0.02	32.28 ± 0.02	32.03 ± 0.05	32.0 ± 0.05

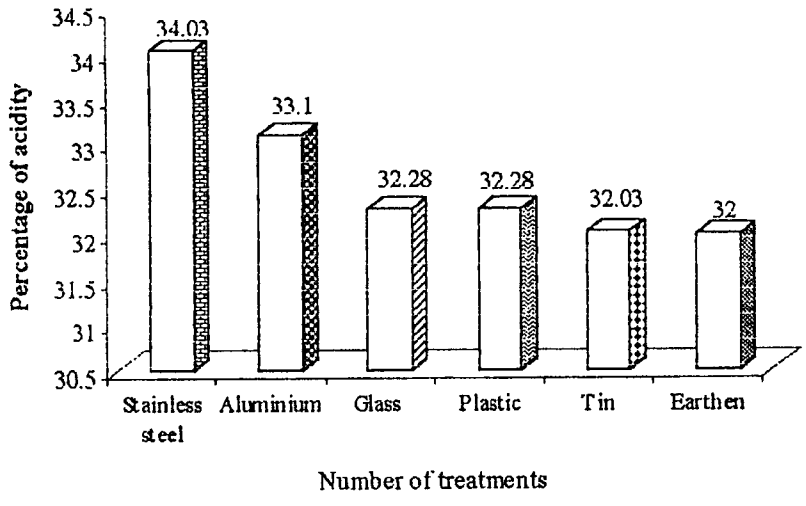

Fig. 4.4 Result of clot-on-boiling time in different containers with storage time in winter season

Table 4.10 Result of Alcohol test in different containers with storage time in winter season

Period of storage (hours)	Stainless steel container	Aluminium container	Glass container	Plastic container	Tin container	Earthen container
0-30th hour	-	-	-	-	-	-
31th hour	-	-	-	-	-	-
32th hour	-	-	+	+	+	+
33th hour	-	+	+	+	+	+
34th hour	+	+	+	+	+	+
Average (COB) Positive time	33.46±0.0	32.45±0.02	32.03±0.05	32.03±0.05	31.46±0.07	31.45±0.05

Table 4.11 Acidity of milk in different containers changes with storage time during winter season

Period of storage (hours)	Stainless steel container	Aluminum container	Glass container	Plastic container	Tin container	Earthen container	LSD value
0-0th hour	0.145	0.145	0.145	0.145	0.145	0.145	
2nd hour	0.145	0.145	0.145	0.145	0.145	0.145	
4th hour	0.145	0.145	0.145	0.145	0.145	0.145	
6th hour	0.145	0.145	0.145	0.146	0.150	0.150	
8th hour	0.145	0.145	0.145	0.146	0.150	0.150	
10th hour	0.145	0.145	0.146	0.148	0.151	0.151	
12th hour	0.150	0.151	0.154	0.148	0.151	0.156	0.004
14th hour	0.150	0.151	0.154	0.150	0.151	0.156	
16th hour	0.150	0.151	0.154	0.150	0.151	0.156	
18th hour	0.150	0.153	0.155	0.153	0.155	0.158	
20th hour	0.150	0.153	0.155	0.153	0.155	0.158	
22th hour	0.150	0.153	0.155	0.153	0.155	0.158	
24th hour	0.150	0.153	0.155	0.153	0.155	0.158	
26th hour	0.155	0.156	0.156	0.160	0.160	0.163	
28th hour	0.156	0.163	0.163	0.166	0.171	0.171	
30th hour	0.173	0.183	0.175	0.180	0.186	0.178	
32th hour	0.190	0.199	0.196	0.196	0.204	0.200	
34th hour	0.212	0.219	0.217	0.219	0.228	0.227	

Table 4.13 Average acidity changes with storage time during winter season

Time (Hours)	% Acidity changes	Ranked
T_0 hours	0.145±0.0	J
T_2 hours	0.145±0.0	J
T_4 hours	0.145±0.0	J
T_6 hours	0.147±0.002	I
T_8 hours	0.147±0.002	I
T_{10} hours	0.148±0.002	II
T_{12} hours	0.152±0.002	G
T_{14} hours	0.152±0.002	G
T_{16} hours	0.152±0.002	G
T_{18} hours	0.154±0.002	F
T_{20} hours	0.154±0.002	F
T_{22} hours	0.154±0.002	F
T_{24} hours	0.154±0.002	F
T_{26} hours	0.158±0.003	E
T_{28} hours	0.165±0.005	D
T_{30} hours	0.179±0.004	C
T_{32} hours	0.197±0.004	B
T_{34} hours	0.220±0.006	A
Level of sig.	**	
LSD Value	0.0002	

Microbiological parameters

Total viable bacteria and coliform bacteria before starting the experiment was 41000 CFU/ml and 110 CFU/ml respectively. During storage period only total viable bacterial population was determined for each container when COB test gave positive result. It was found that the total viable bacterial population of milk

samples were 100000 CFU/ml, 125000 CFU/ml, 125000 CFU/ml, 125000 CFU/ml, 150000 CFU/ml and 15500000 CFU/ml in stainless steel, aluminium, glass, plastic, tin and earthen containers respectively.

From the result of both phases of study it was found that stainless steel container was found best among other containers for storage of milk. Earthen container was worst. Performance of tin container was similar to earthen container. Glass and plastic containers was better than tin and earthen container but inferior than aluminium container. Earthen container have small pores and through these pores microorganisms can easily pass into milk and helps quick fermentation of lactose by secreting lactase enzyme, as a result acidity increased rapidly in earthen container. Similarly tin containers had some metalic effects which helped to spoilage milk. Although glass and plastic containers showed nearly similar performance, but organoleptically glass container seems better than plastic container. Plastic had got some off-flavour which is absent in glass container. If milk is to be kept in glass container then it should be protected from slulight. Otherwise fat will oxidize and will dcvclop objcctionablc flavour. Stainlcss stccl containcr had no catalytic cffccts on milk. So, quality of milk was best in stainless steel container.

Regarding season it was found that temperature had some influence on storage life of milk. During summer season temperature ranges from 32 to 34^{0}C which was favourablc for thc growth of acid producing bactcria but in wintcr scason temperature was around 18 to 20^{0}C which was not favourable for acid producing bacteria. As a result acidity increased rapidly in summer season than winter season, which had created difference in keeping quality regarding time. For this reason milk sample did not spoil early in winter than summers season.

Judging from the result of all parameters studied it was found that stainless steel container is best for storage of milk in any season of the year and next is aluminium

container. Earthen and tin containers are worst regarding keeping quality. In summer season when temperature is about 32 to 34^{0}C then high quality raw milk could be kept in good condition upto 10-12 hours in any type of container and in winter season similar type of milk could be kept for about 30 hours in any type of container when room temperature ranged from 18-22^{0}C.

SUMMARY AND CONCLUSION

The main objective was to study the effect of different container on the storage life of milk at room temperature. To achieve the stated objectives the research was divided into two phases.

Phases 1 Six different types of containers (stainless steel, aluminium, glass, plastic, tin and earthen pot) were used to preserve milk at room temperature during summer season. The room temperature ranged from 31.1-31.7^{0}C during the storage period. The physical, chemical and microbiological tests were done initially and then the quality of milk samples were measured at every two hours interval from each container upto 12 hours and thereafter every one hour interval until spoilage to asses the quality of milk. It was found that the percentage of acidity increased with storage time in each container. The percentage of acidity was within the normal range upto 12-hours and milk was found good upto that period and thereafter acidity increased rapidly in earthen and tin containers and slowly in stainless steel and aluminium containers. Milk was found in good condition 15 hours in stainless steel container, 14 hours in aluminium, glass and plastic, 12 hours in tin and earthen containers respectively. Thereafter with storage time milk was spoilage rapidly in tin and earthen container. The acidity percentage of all other containers were in between stainless steel and tin container. The COB and alcohol tests confirmed the results of acidity test. The COB test were 15.30±0.15, 14.30±0.15, 14.30±0.15,

14.0±0.15, 12.0±0.15, 12.0±0.03 and 15.03±0.05, 14.03±0.05, 14.08±0.07, 13.28±0.02, 11.30±0.05, 11.32±0.02 in stainless steel, aluminium, glass, plastic, tin and earthen containers respectively. This result is in agreement with Mahboob *et al.* (1992).

In the 2nd phase of the experiment, the similar trend of result was observed in different types of container in winter season also. During starting the experiment, the initial acidity was 0.145 in all the containers and the room temperature was 19^{0}C. The acidity of all containers remain unchanged upto 10th hours in all the container except tin and earthen container where acidity was 0.151and the temperature ranged from 19 to 21^{0}C at that time. Then the acidity slightly increased in all the container at 12th hour of experiment. These percentage of acidity was stil unchanged upto 24th hours of experimental period due to lowering of room temperature at night which ranged from 20-18^{0}C. Then the percentage of acidity increased gradually with storage time in each container (table 10). The COB and alcohol tests also confirmed the result of acidity. Statistical analysis showed that there was significant difference ($P<0.01$) between containers in terms of keeping quality of milk.

It may be concluded that both the season or around the year container had positive effect for keeping milk and stainless steel container was found best, the earthen and tin container was bad/worst container in all respect in terms of keeping quality of milk.

Expt. 5. Effect of banana leaf on the shelf-life of milk

INTRODUCTION

In Bangladesh, the milk business man during transporting milk from village to city areas used to put banana leaf on the top of their milk containers. In most cases they keep about 30 litres of milk in each container and the top of the container is open. Their general believe is that banana leaf prevents agitation and also increases the shelf life of milk. Agitation usually separates fat from milk. If they can stop agitation, that will help to keep milk in good condition. So, putting banana leaf on the top of milk containers might have some scientific effects for preventing agitation. But the effect on shelf life of milk is questionable. As milk vendors believe that this leaf helps to increase the shelf life of milk, for this reason it is important to investigate this matter. Hence, in order to obtain idea where the banana leaf has any effect on the shelf-life of milk, the present experiment was conducted.

MATERIALS AND METHODS

3.5.2.1 Place of experiment

The experiment was conducted at the Dairy Technology and Dairy Microbiology Laboratory of the Department of Dairy Science, Bangladesh Agricultural University, Mymensingh.

3.5.2.2 Collection of milk samples

Milk samples were collected from Bangladesh Agricultural University Dairy Farm. In each experimental day, after collection of sample, they were transferred immediately to the Laboratory for analytical purpose.

3.5.2.3 Collection of banana leaf

Banana leaf were collected from farmers house of surrounding villages of University Campus. In each time same type of leaf were collected. After collected leaf were washed with water and cutted into small pieces before putting on milk samples.

3.5.2.4 Experimental procedure

The collected milk samples were analyzed to know their initial quality. The parameter used to monitor the initial quality of milk were fat content, SNF and total-solids content, acidity percentage and specific gravity of milk samples.

The collected milk samples were divided into three portion and each portion of milk was kept in separate aluminum containers. Aluminum container was selected for storage of milk, as because in our one of the previous experiment it was found that stainless steel and aluminum containers were good for storage of milk. As aluminum container is very cheap and available throughout the country, for this reason aluminum container was selected.

After keeping milk in three containers, one was kept as it is without putting banana leaf on the top, but 2 and 3 per cent chopped banana leaf on the basis of weight of samples were put on the top of other two containers respectively. So, finally there were three treatment, one without banana leaf (control) and designated as T_1, another containing 2 per cent banana leaf and designed as T_2, the remaining third container containing 3 per cent banana leaf was treated as T_3 group.

They are shown below:

T_1 = Only milk in the container
T_2 = Milk + 2 per cent banana leaf
T_3 = Milk + 3 per cent banana leaf

The three containers were kept at room temperature in the laboratory until clots. The following parameters were studied during the storage period to judge the quality of milk and also to monitor the effectiveness of banana leaf as milk preservatives.

3.5.2.5. The parameters were

i) Acidity test (%)

ii) Clot-on-boiling test (COB)

3.5.2.6 Data analysis

Data collected on different parameters were summarized and when necessary were analyzed statistically by using Completely Randomized Design (CRD) as per Steel and Torric (1980). If necessary LSD test was also performed.

RESULTS AND DISCUSSION

3.5.3.1 Initial quality of milk

Results of initial quality of milk is shown in Table 5.1. From the table it is evident that all parameters of milk collected from Bangladesh Agricultural University Dairy Farm were within normal range. So, good quality milk was used in this experiment.

Table 5.1 The initial quality of milk at the begining of the experiment

Sl. No.	Parameters	Fresh milk
1.	Specific gravity	1.028±0.0005
2.	Acidity per cent	0.153±0.0
3.	Fat (g/kg)	38±0.15
4.	S.N.F (g/kg)	77.6
5.	T.S. (g/kg)	115.6

3.5.3.2 Storage study

i) Acidity test

The acidity per cent of control and banana leaf treated milk samples were determied on every one hour interval starting from 'O' hour to 15 hours. The result of acidity changes in milk samples are shown in (Table 5.2. and Fig. 5.1) From the Table it is observed that initial acidity of T_1, T_2 and T_3 groups were same (0.153%). But with the progress of storage time acidity of sample started to increase gradually. But for T_2 and T_3 were where banana leaf were added, acidity decreases slightly upto 2

hours and there after started to increase slightly rapidly than T_1 samples. Acidity of all three samples were acceptable upto 12 hours and thereafter that the values were not accepted level which was confirmed by clot-on-boiling test, (Table 5.3). Statistical analysis showed that there was no significant differene within average acidity of three samples.

The result of acidity test indicated that there was no benefit of adding banana leaf for enhancing the shelf life of milk. Decrease in acidity during first two hours might be due to the fact that banana leaf is alkaline in nature and for this reason alkaline material secreted from banana leaf might have influenced to decrease the acidity level up to some extent but after two hours this effect was not noticed. Moreover, the increase in acidity was little faster than the control milk samples. All three milk samples become unfit at 13 hours which indicated that banana leaf might be useful for preventing agitation but not for increasing the shelf life of milk.

When the acidity of banana leaf added samples decreased up to 2 hours it was expected that this leaf might have some effect on storage life. But at the end of the study it was not found. This possible reason for that the green leaf cells might have oxidized and generated some heat in milk which might be a cause of faster acid production in milk. The result of acidity production with storage time agrees with the work of lot of workers Ahad (1997) and Rafique (1998). This is due to the break down of lactose by Lactic acid producing bacteria with the help of lactase enzyme secreted by them.

Table 5.2 Average acidity changes with storage time during experimental period

Time (hours)	Fresh milk (control) (T_1)	2 per cent Banana leaf with milk (T_2)	3 per cent Banana leaf with milk (T_3)
0^{th} hour	0.153	0.153	0.153
1^{st} hour	0.153	0.152	0.153
2^{nd} hour	0.154	0.151	0.151
3^{rd} hour	0.154	0.154	0.156
4^{th} hour	0.155	0.157	0.159
5^{th} hour	0.156	0.160	0.162
6^{th} hour	0.158	0.163	0.165
7^{th} hour	0.161	0.167	0.169
8^{th} hour	0.168	0.173	0.175
9^{th} hour	0.175	0.180	0.182
10^{th} hour	0.182	0.188	0.190
11^{th} hour	0.190	0.197	0.199
12^{th} hour	0.201	0.207	0.209
13^{th} hour	0.215	0.215	0.218
Mean ± SD	0.169±0.020	0.173±0.021	0.174±0.022
Level of Significance	NS		

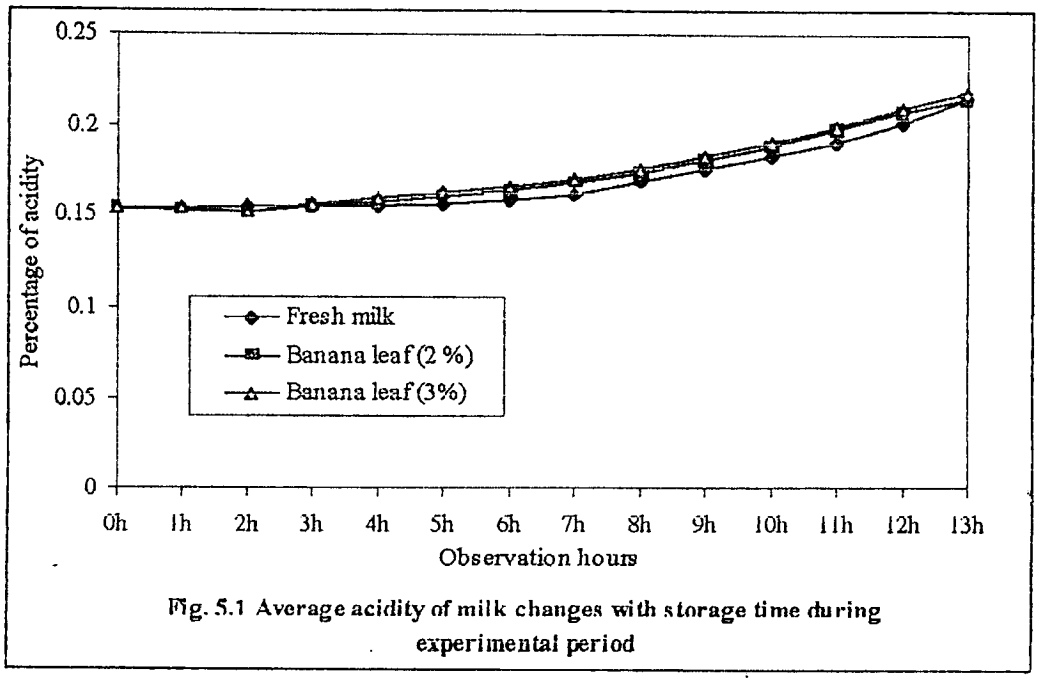

Fig. 5.1 Average acidity of milk changes with storage time during experimental period

ii) Clot-on-boiling test

Clot-on-boiling test was done to monitor the result of acidity test. When the acidity level of milk exceeds the desired level then milk clots if heat is applied on them. In this trial clot-on-boiling test gave negative result up to 11^{th} hour in all groups but gave positive result at 12^{th} hour. This means both treated and untreated milk samples become unfit for further processing at 12^{th} hour of study. The result of COB test supports the result of acidity test. This findings also suggested that banana leaf is not an effective material for enhancing the shelf-life of milk although this leaf contains alkaline material. The result of this study are in agreement with the findings of Biswas (1997) who in a preliminary study found that banana leaf could not increase the shelf-life of milk.

Table 5.3 Average clot-on-boiling time of milk during the study period.

Hour after treatment	Treatments		
	T_1	T_2	T_3
$0–6^{th}$ hour	–	–	–
7^{th} hour	–	–	–
8^{th} hour	–	–	–
9^{th} hour	–	–	–
10^{th} hour	–	–	–
11^{th} hour	–	–	–
12^{th} hour	+	+	+

SUMMARY AND CONCLUSION

The present experiment was conducted to monitor the effectiveness of adding banana leaf on milk as short time preservative of milk. For this purpose milk samples were collected from Bangladesh Agricultural University Dairy Farm and fresh green banana leaf were collected from village farmer's house. The collected milk samples were analysed initially to see their quality and there after divided into three equal parts having approximately one litre of milk in each part. The milk samples were kept in an aluminium pot. Banana leaf were cutted into small pieces in the laboratory. Pieces of leaf were put on the top of milk in two containers at the rate of 2 and 3 per cent of the weight of milk. Another container was kept without banana leaf and was treated as control group. All three type of samples were stored at room temperature in laboratory until COB test gave positive result.

From the result it was found that initial quality of milk was in good condition. To measure the quality of milk during storage period, acidity (percent) and COB test was conducted. From the result of both parameters it was found that all milk samples was good condition upto 11 hours. There after they gave COB positive test which indicated that enough developed acidity was formed in milk which was responsible for clotting of milk. So, from this findings it can be mentioned that banana leaf have no preservative effect although village farmers' believe it but it helps to prevent agitation of milk, thus save milk from fat separation.

Experiment 6: Effect of added water on storage life of milk

INTRODUCTION

In is well known that milk is a perishable product. Village farmers' have not enough technology to prevent milk from spoilage. After purchaging milk from market they used to boil milk at home on open fire. But under some unavoidable circumstances they also add some portion of cold water with milk to keep it safe for a while. They practiced this things in their own home not for selling in the market. But some bad people used to sell milk by adding water with it. Our aim was to monitor the effectiveness of adding water on shelf-life of milk. Hypothetically their might have some effects as because water will decrease the temperature of milk and acidity will also be decreased. Keeping this things in mind, the present study was carried out.

MATERIALS AND METHODS

Similar to other experiments, this was also done at the Dairy Technology and Dairy Microbiology Laboratory of the Department of Dairy Science, Bangladesh Agricultural University, Mymensingh.

3.6.2.1 Collection of milk samples

Fresh milk samples were collected from Bangladesh Agricultural University Dairy Farm. After collected samples were analyzed to judge their initial quality. Parameter used to monitor the initial quality of milk were fat, SNF and total-solids content, acidity and specific gravity of milk.

3.6.2.2 Experimental procedure

Collected milk samples were divided into four equal portions. One portion was kept as it is an aluminium container in the laboratory at room temperature but in other three samples 10, 20 and 30 per cent water was added respectively. All of them were kept in similar aluminum containers and were stored at room temperature of

the laboratory like control milk samples. Finally four samples were obtained and these were shown below.

1) Whole milk

2) Whole milk + 10 per cent water added

3) Whole milk + 20 per cent water added

4) Whole milk + 30 per cent water added

3.6.2.3 Parameters studied

The following tests were done to judge the quality of milk during storage time

(i) Acidity of milk per cent
(ii) COB test

Both of the tests were done as per method described by Aggarwala and Sharma (1962).

2.6.2.4 Data analysis

Collected data were subjected to statistical analysis. Analysis of variance test was done by using one way analysis of variance test as per Steel and Torrie (1980).

RESULTS AND DISCUSSION

3.6.3.1 Initial quality of milk

The initial quality of milk is shown in Table 6.1. From the Table it is evident that Fat, SNF, Total-Solids content of milk samples were 41.0, 83.9 and 125.0 g/kg respectively. The values were within normal range. On the other hand acidity was 0.159% and specific gravity was 1.029. This two values were also within normal range. So, its initial results indicates that quality of milk samples used in this experiment were normal. After adding water, values of all parameters decreased slightly which was shown in Table 6.1.

Table 6.1 Initial quality of milk at the begining of the experiment

Parameters	Type of milk			
	Whole milk	Whole milk + 10 per cent water	Whole milk + 20 per cent water	Whole milk + 30 per cent water
Fat (g/kg)	41.0	39.5	31.0	26.0
SNF (g/kg)	83.9	77.5	70.3	62.5
Total-Solids (g/kg)	125.0	11.70	101.3	86.60
Acidity per cent	0.155±0.0	0.139±0.0	0.130±0.0	0.118±0.0
Specific gravity	1.029±0.0005	1.027±0.0005	1.023±0.0005	1.020±0.0005

3.6.3.2 Storage study

The quality of milk and storage time were determined with the help of acidity and COB test. All these tests were done initially at '0' hours and thereafter every one hour interval until the COB test gave positive result.

i) Acidity test

Initial acidity of fresh milk (control), 10, 20 and 30 per cent water added samples were 0.115, 0.139, 1.130 and 0.118 per cent respectively. The acidity of all samples increased gradually with storage time but the increment was little faster in whole milk samples. Room temperature during the study period was about 29^{0}C. In fresh milk sample acidity was acceptable level upto 11 hours and for 10, 20 and 30 per cent water added samples the figure were 12, 13 and 15 hours respectively. This indicated that addition of water increased the shelf-life of milk. This effects may be of two types, firstly addition of cold water decreases the temperature of milk and secondly it decreased acidity of milk. Statistical analysis showed that the mean acidity of water mixed samples were significantly lower than that of control milk samples ($P<0.01$).

The result of acid production agrees with the work of several workers. Bogdanova *et al.* (1976) found that acidity increased with storage time. Duncan *et al.* (1991) also reported that average daily acidity increased with storage time. Islam (1984) observed that acidity of Bangladesh Agricultural University Dairy Farm milk was 0.15 per cent and that of MM was 0.14 per cent. Ghafoor *et al.* (1985) mentioned that at 30–35^{0}C, acidity upgrade from 0.15 to 0.75% after 12 hours and they also recommended that in July and August milk can be stored for 4 hours without any deterioration in quality. Dehury *et al.* (1977) showed that milk acidity collected from established dairy farm, local gowalas, local shops, milk collection centers and pasteurized milk booths were 0.15, 0.12, 0.11, 0.15 and 0.14 per cent. El-Shazla *et al.* (1978) reported that acidity per cent of milk collected from private herds was 0.188±0.02% (mean±SD). Nakae *et al.* (1978) interpreted that acidity of milk was 0.13 to 0.15 per cent. Yadav and Sarawat (1982) said that the market milk sample had higher acidity. Jurgens (1983) showed that low acidity values are in themselves an indicator of possible watering. Bonczar and Gardzina (1986) proved that acidity is better indicator of watering than coagulation time which varied widely from cow to cow. Bae *et al.* (1992) found in his experiment that acidity per cent unclean water used farm's were higher than clean water used dairy farms. Reddy *et al.* (1989) cited that bulk farm tank raw milk stored for 0, 3 and 6 hour respectively at 25–30^{0}C temperature showed 0.162, 0.177 and 0.194 per cent titratable acidity.

Table 6.2 Average acidity changes with storage time during experimental period

Time (hours)	Whole milk	Whole milk + 10 per cent water	Whole milk + 20 per cent water	Whole milk + 30 per cent water
0th hour	0.155	0.139	0.130	0.118
1st hour	0.155	0.139	0.130	0.118
2nd hour	0.155	0.139	0.130	0.118
3rd hour	0.155	0.139	0.130	0.118
4th hour	0.156	0.143	0.133	0.122
5th hour	0.156	0.145	0.138	0.125
6th hour	0.158	0.147	0.140	0.132
7th hour	0.161	0.150	0.143	0.139
8th hour	0.168	0.156	0.146	0.146
9th hour	0.175	0.161	0.155	0.151
10th hour	0.182	0.175	0.165	0.159
11th hour	0.190	0.182	0.178	0.165
12th hour	0.201	0.190	0.191	0.175
13th hour	0.215	0.201	0.199	0.189
14th hour	0.236	0.212	0.205	0.195
15th hour	0.250	0.225	0.215	0.205
16th hour	0.270	0.250	0.240	0.220
Mean ± SD	0.185±0.04	0.170±0.05	0.163±0.04	0.153±0.03
Level of Significance	**			

** = Significant at 01 per cent level

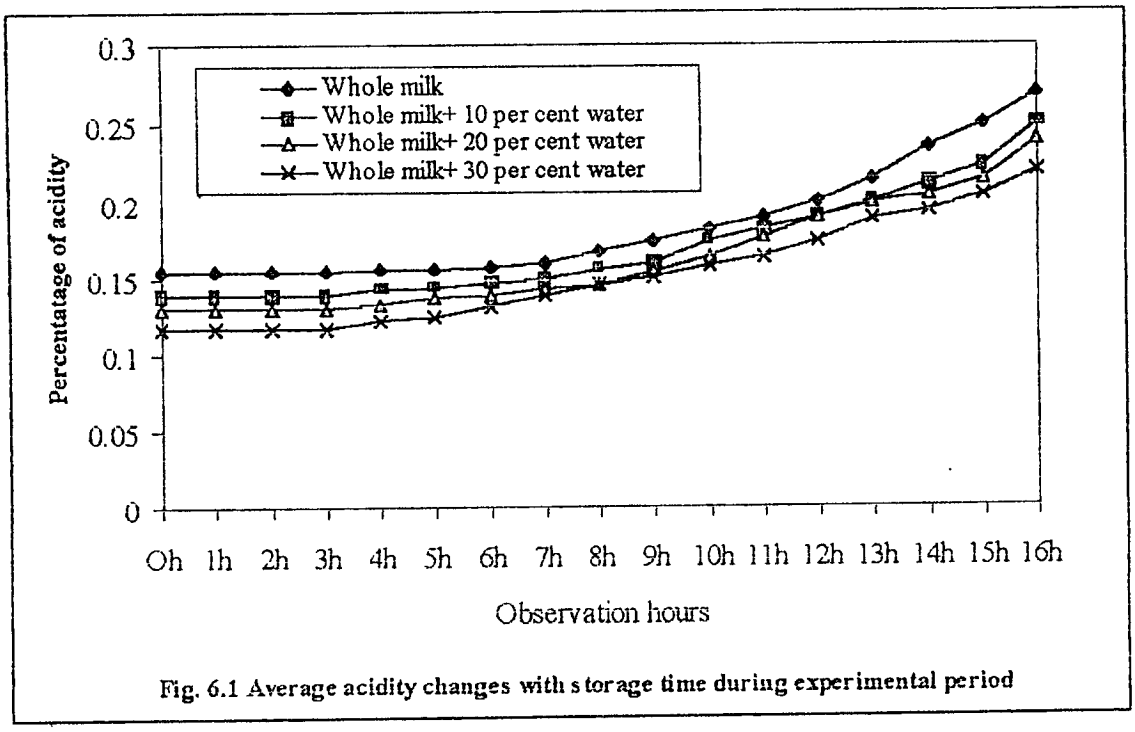

Fig. 6.1 Average acidity changes with storage time during experimental period

ii) Clot-on-boiling test

Clot-on-boiling test was done in the laboratory by heating small amount of milk in a test tube. If clotting or tendency of clotting was noticed, the result was considered as positive. This test was done to check the accuracy of the result of acidity test. So, result of both the tests indicated that shelf-life of milk could be increased for a while by mixing water in it.

Table 6.3 Average clot-on-boiling time of milk during the study period.

Hour after treatment	Treatments			
	Whole milk	Whole milk + 10 water	Whole milk + 20 water	Whole milk + 30 water
0–8th hour	–	–	–	–
9th hour	–	–	–	–
10th hour	–	–	–	–
11th hour	–	–	–	–
12th hour	+	–	–	–
13th hour	+	+	–	–
14th hour	+	+	+	–
15th hour	+	+	+	–
16th hour	+	+	+	+

SUMMERY AND CONCLUSION

In this experiment attempts were made to evaluate the effects of adding cold fresh water on shelf-life of milk. For this purpose 10, 20 and 30 per cent of fresh cold water collected from hand operated tube well were added with milk. One portion of milk was taken without adding water. Finally four different types of samples were obtained. These were i) Milk samples without added water, ii) Milk samples with 10 per cent added water, iii) Milk samples with 20 per cent added water and iv) Milk samples with 30 per cent added water. All these samples were taken separately in aliminium containers and kept at room temperature of the Laboratory for a period of sixteen hours. Parameters used to monitor the storage period of milk were acidity and COB test. From the result of both acidity and COB test it was observed that fresh milk could be stored upto 11 hours where as 10, 20 and 30 per cent water added samples could be stored upto 12, 13 and 15 hours respectively in acceptable condition when the room temperature was approximately 29^0C. From this result it can be suggested that addition of cold water in milk is effective to enhance the shelf-life of milk. But while doing this we have to keep in mind that practice could only be done in household condition for own consumption but not for selling in the market. It any body do this, that would be treated as an adulteration.

CHAPTER 4

GENERAL DISCUSSION AND CONCLUSION

Chapter 4

GENERAL DISCUSSION AND CONCLUSION

This research project was carried out to evaluate the quality of market milk collected from selected areas of Bangladesh and also to monitor the feasibility of using existing traditional technologies used by farmers' for increasing the shelf-life of milk under village conditions. At the same time attempts were also made to find out a suitable technology of milk preservation for rural condition. For this purpose a series of six experiments were conducted, out of which two were related with milk quality and another four were related to milk preservation.

First experiment, regarding milk quality was carried in Mymensingh district. Milk samples were collected from for different places, namely Bangladesh Agricultural University Dairy Farm, Mymensingh Town, local village markets and directly from village farmers'. The parameters used to monitor the quality of milk samples were i) Physical (colour, taste, flavour and specific gravity), ii) Chemical (acidity, fat, SNF, TS, protein, lactose and ash content) and iii) Microbiological (total viable bacterial count and coliform count). A total of 120 samples were analyzed taking 30 samples from each of the above mentioned places. From organoleptic evaluation it was found that colour of all milk samples collected form Bangladesh Agricultural University Dairy Farm and Mymensingh Town was normal (golden yellowish white), but for local markets and village farmers' milk 33.33 and 13.33 per cent samples gave light golden yellowish white colour. Taste of 100 per cent milk samples from Bangladesh Agricultural University Dairy Farm milk was normal but for Mymensingh Town milk 10 per cent milk gave flat taste and remaining 90 per cent milk gave normal taste. In case of local market and village farmers' samples, 70 and 86.67 per cent samples gave normal taste, but 30 and 13.33 per cent samples gave flat taste respectively. Flavour of all samples collected from Bangladesh Agricultural University Dairy Farm was normal but for Mymensingh Town milk 90 and 10 per cent milk gave normal and non-milky flavour respectively. In case of local markets and village farmers' milk 83.33 and 90 per cent samples gave normal

flavour but 16.67 and 10 per cent samples gave non-milky flavour respectively. From the organoleptic evaluations it can be mentioned here that quality of milk collected from Bangladesh Agricultural University Dairy Farm was better than other samples. Slightly abnormal flavour and taste of other samples might be due to addition of little water and non-scientific milking procedure followed by milkers' and unhygienic practies of milk businessman during handling and transportation of milk. On the other hand it may be mentioned that the colour, taste and flavour of milk varies depending on may factors such as breeds of animal, the kind of feed offered, the amount of fat and solids present in milk. Average specific gravity of all four type of milk samples were within normal range but some samples gave lower specific gravity. Lower specific gravity of milk indicates that the quality of milk is inferior which may be due to adulteration of water. Milk fat has some influence on the specific gravity of milk also. Regarding chemical parameters it was found that average fat, SNF, TS, protien lactose and ash (g/kg) of milk samples collected from different places of Mymensingh district were 46.267±4.48, 33.533±6.48, 35.867±5.42, 40.3±6.09; 82.98±3.47, 72.184±15.86, 75.733±8.97, 80.345±6.44; 128.580±6.86, 104.073±22.75, 111.530±10.49, 120.717±10.18; 35.10±0.92, 34.733±1.26, 35.233±0.77, 35.433±0.77; 40.947±3.42, 32.823±9.19, 33.733±8.59, 37.697±6.86 and 6.9±0.2, 6.6±1.14, 6.8±0.23, 6.9±0.10 g/kg respectively. From the analysis of adulteration it was found that no water was added in Bangladesh Agricultural University Dairy Farm milk but in case of Mymensingh Town and Local markets samples about 12.52 and 11.09 per cent water was added. But in case of village farmers' milk about 5.83 per cent of added water was detected which is very small amout and might be from washing of utensils with water which are same timers left in milking cans. Bacteriological study indicated that strict hygienic conditions were not maintained by milk producers. For this reason both viable bacterial count and coliform count for all samples were above normal values.

The second experiment was conducted in northern part of Bangladesh to study the quality of milk. Milk samples were collected from four different places, namely, i)

Sirajgonj Town, ii) Local markets of Sirajgonj district, iii) Village markets of Pana (boarder of Sirajgonj) and iv) Milk shed area of Pabna district). Parameters used to monitor the quality of milk were similar to the first experiment. No variations were noticed in colour, taste and flavour of milk samples collected from four different sources except Sirajgonj Town milk where only 15 per cent samples gave flat taste. Average specific gravity of all milk samples were within normal range. Chemical analysis showed that average milk fat, SNF, and T.S (g/kg) of milk collected from four different places such as Sirajgonj town, local market of Sirajonj, village marked of Pabna and milk shed area of Pabna (society) were 3.15+4.97, 37.10±2.27, 43.25±3.10, 43.25±2.07; 78.05±11.32, 75.17±1.96, 80.90±2.37, 77.27±2.41 and 109.56±15.23, 112.26±2.01, 124.15±2.62, 120.54±3.49 gm/kg respectively. Statistical analysis showed that the differences between fat and TS content of milk samples were significant ($P<0.01$) at 1 per cent level except SNF content of milk samples which was found significant ($P<0.05$) at 5 per cent level. Small amount of added water was detected from all four types of samples. The percentage of added water were 9.19, 11.53, 4.78 and 9.05 per cent in the milk of Sirajgonj town, local market of Sirajonj, village marked of Pabna and milk shed area of Pabna (society) respectively. Bacteriology study also showed that total number of viable and coliform bacteria were higher than normal level in all samples. This indicated that hygienic condition of milking was not adequate.

The third experiment was carried out to know the effect of soduim bicarbonate on the keeping quality of milk. For this purpose, milk samples were collected from BAU Dairy Farm and taken to the laboratory for analsysis. The physical, chemical and microbiological qualities of milk were determined before adding sodium bicarbonate with milk samples. Organoleptic test was performed nasally, visually to observe colour, flavour and texture of milk. The colour, taste, flavour and texture of milk samples were golden yellowesh white (100%), slightly sweet (100%), mild aromatic (100%) and free flowering liquid (100%). Thereafter collected milk samples were preserved at room temperature (32-34°C) with 0.10, 0.150 and 0.20

percent sodium bicarbonate. One group was kept without sodium bicarbonate and was considered as control group. The quality of milk samples were measured at every two hours interval upto 12 hours and thereafter every one hour interval until spoilage to asses the quality of milk. Initially, colour, flavour and texture of all milk samples were normal (100%), but with progressive storage time colour, flavour and texture of all samples deteriorated gradually. The deterioration was more rapid for control samples than that of the sodium bicarbonate treated samples. Acidity percent of all samples increased gradually during storage period and the differences in acidity of milk samples in different treatments were significant ($P<0.01$). Increased was significantly ($P<0.01$) more in control samples than that of the treated samples. The result of acidity test was supported by COB test. Control samples spoiled after 12 hours but that of 0.10, 0.15 and 0.20 percent sodium bicarbonate treated samples spoiled after 13,14 and 16 hours respectively. It may be concluded that $NaHCO_3$ is the effective chemical for neutralizing the acids produced by acid producing bacteria and can be used for a short term preservation of milk under rural condition of Bangladesh where scientific cooling or pasteurization facilities are not available.

The fourth experiment was conducted to study the effect of different container on the storage life of milk at room temperature. To achieve the stated objectives the research was divided into two phases.

In the first phase, six different types of containers (stainless steel, aluminium, glass, plastic, tin and earthen pot) were used to preserve milk at room temperature during summer season. The room temperature ranged from 31.1-31.7^{0}C during the storage period. The physical, chemical and microbiological tests were done initially and then the quality of milk samples were measured at every two hours interval from each container upto 12 hours and thereafter every one hour interval until spoilage to asses the quality of milk. It was found that the percentage of acidity increased with storage time in each container. The percentage of acidity was within the normal range upto 12-hours and milk was found good upto that period and thereafter

acidity increased rapidly in earthen and tin containers and slowly in stainless steel and aluminium containers. Milk was found in good condition upto 15 hours in stainless steel container, 14 hours in aluminium, glass and plastic, 12 hours in tin and earthen containers respectively. Thereafter with storage time milk spoiled rapidly in tin and earthen container. The acidity percentage of all other containers were in between stainless steel and tin container. The COB and alcohol tests confirmed the results of acidity test. The COB test gave positive result at 15.30±0.15, 14.30±0.15, 14.30±0.15, 14.0±0.15, 12.0±0.15 and 12.0±0.03 hours, whereas alcohol test gave positive result at 15.03+0.05, 14.03+0.05, 14.08+0.07, 13.28±0.02, 11.30±0.05 and 11.32±0.02 hours respectively in stainless steel, aluminium, glass, plastic, tin and earthen containers.

In the second phase of the experiment, the same thing was repeated in winter season to see whether the effects of containers varied or not depending on season of the year. During starting the experiment, the initial acidity was 0.145 in all the containers and the room temperature was 19^{0}C. The acidity of all containers remain unchanged upto 10th hours in all the container except tin and earthen container where acidity was 0.151 per cent and the temperature ranged from 19 to 21^{0}C at that time. Then the acidity slightly increased in all the container. These percentage of acidity was stil unchanged upto 24th hours of experimental period due to lowering of room temperature at night which ranged from 20-18^{0}C. Thereafter the percentage of acidity increased gradually with storage time in each container. The COB and alcohol tests also confirmed the result of acidity. Finally it was observed that milk samples were in acceptable condition upto 34.03, 33.10, 32.28, 32.28, 32.03 and 32.0 hours in stainless steel, aluminium, glass, plastic, tin and earthen containers respectively. This result indicated that shelf-life of milk was very high in winter season but effects of containers were similar both in summer and winter.

It may be concluded that both the season or around the year container had positive effect for keeping milk and stainless steel container was found best, the earthen and

tin container was bad/worst container in all respect in terms of keeping quality of milk and also could delay clotting time of milk.

The fifth experiment was conducted to monitor the effectiveness of adding banana leaf on milk as short time preservative. For this purpose milk samples were collected from Bangladesh Agricultural University Dairy Farm and fresh green banana leaf were collected from village farmer's house. The collected milk samples were analysed initially to see their quality and there after divided into three equal parts having approximately one litre of milk in each part. The milk samples were kept in an aluminium pot. Banana leaf was cutted into small pieces in the laboratory. Pieces of leaf were put on the top of milk in two containers at the rate of 2 and 3 per cent of the weight of milk. Another container was kept without banana leaf and was treated as control group. All three type of samples were stored at room temperature in the laboratory until COB test gave positive result. From the result it was found that initial quality of milk was in good condition. To measure the quality of milk during storage period, acidity (percent) and COB test was conducted. From the result of both parameters it was found that all milk samples was good condition upto 11 hours. There after they gave COB positive test which indicated that enough developed acidity was formed in milk which was responsible for clotting of milk. So, from this findings it can be mentioned that banana leaf have no preservative effect although village farmers' believe it but it helps to prevent agitation of milk, thus save milk from fat separation.

The six experiment was carried out to evaluate the effects of adding cold fresh water on shelf-life of milk. For this purpose 10, 20 and 30 per cent of fresh cold water collected from hand operated tube well were added with milk. One portion of milk was taken without adding water. Finally four different types of samples were obtained. These were i) Milk samples without added water, ii) Milk samples with 10 per cent added water, iii) Milk samples with 20 per cent added water and iv) Milk samples with 30 per cent added water. All these samples were taken separately in aliminium containers and kept at room temperature of the Laboratory for a period

of sixteen hours. Parameters used to monitor the storage period of milk were acidity and COB test. From the result of both acidity and COB test it was observed that fresh milk could be stored upto 11 hours where as 10, 20 and 30 per cent water added samples could be stored upto 12, 13 and 15 hours respectively in acceptable condition when the room temperature was approximately 29^0C. From this result it can be suggested that addition of cold water in milk is effective to enhance the shelf-life of milk. But while doing this we have to keep in mind that this practice could only be done in household condition for own consumption but not for selling in the market. If any body do this, that would be treated as an adulteration.

Judging from the results of quality study of milk it might be concluded that wide variations were noticed regarding physical, chemical and microbiological qualities of milk. It appears that quality of milk produced by Bangladesh Agricultural University Dairy Farm was superior to other milk samples. Bacteriological study indicated that proper hygienic conditions were not maintaned during milking and handling milk samples. Regarding shelf-life and milk preservation it could be mentioned that sodium bicarbonate ($NaHCO_3$) could be used as a cheap preservative but that milk should not be used for yoghurt/dahi preparation, as because sodium bicarbonate ($NaHCO_3$) will neutralize the lactic acid and fermentation will be slow. Stainless steel container is good for keeping milk followed by aluminium and others. Banana leaf is not suitable for milk preservation. Addition of 20 to 30 per cent water with whole milk could increase the shelf-life upto three to four hours more than normal shelf-life of whole milk. This practice is only suggested for milk which will be consumed in the family and not to be sold in the market, otherwise it would be an adulteration. The added water could easily be removed by applying heat on milk i.e. by boiling.

Finally, it could be pointed out that the overall findings of this study will be very much helpful for consumers, as well as for rural farmers' who are engaged in milk production.

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APPENDICES

APPENDICES

(i) Organoleptic Test

Organoleptic test was done by the organs of the body like eye, nose, tongue to observe the colour, flavour, texturel consistency/body and taste of the sample. A panel of judge was selected who evaluated the milk samples and scored the sample according to the scorecard.

Each sample was criticized for defects if there was any and remarded for colour, taste, flavour and body/texture/consistency. The samples were placed randomly before judges with the code number.

(ii) Clot-on-boiling (COB) test

About 2 ml of milk was taken in a sterilized test tube and the tube was heated on flame until the milk was boiled. Clotting of milk on boiling indicated that the milk was spoiled and not suitable for pasteurization. On the other hand milk sample which did not clot on boiling indicated that milk was not spoiled and suitable for pasteurization.

(iii) Alcohol test

Place 5 ml of milk in a test-tube and add equal quantity of alcohol. Mix the contents of the test-tube by inverting several times. Note any flakes or clot. The presence of a flake or clot denotes a positive test. A negative test indicates low acidity and good heat stability of the milk sample. Milk showing positive test is not considered suitable for the manufacture of evaporated milk, which has to be sterilized to ensure its keeping quality:

(iv) Determination of fat percentage of milk

The percentage of fat was determined by Babcock Method. 17.6 ml of well-mixed milk sample was taken in a Babcock fat test bottle. Then 17.5 ml of commercial sulphuric acid (Sp. gr. 1.83) were gradually added and mixed until all the constituents of milk except fat were dissolved. The sample was then centrifuged in a

Babcock centrifuge at 140^0 F for 5 minutes. Water at 140^0 F was added upto the neck of the bottle and the sample was centrifuged against for 2 minutes. After this, the fat column was brought in the graduated scale of the bottles by adding water at 140^0 F. The sample was centrifuged again for one minute. The bottle was then placed in a hot water both at 135^0 F. The sample was centrifuged again for one minute. The bottle was then placed in a hot water both at 135^0F to 140^0F for 5 minutes, for raising the fat column in a proper shape. The divider from the lower meniscus to the upper meniscus of he fat column estimated the fat percent.

(v) Determination of acidity

Eighteen-gram (17.6 ml) of milk was taken into a beaker by milk pipette. Five (5) drops of phenolphthalein was added into the milk and was shaken well properly. 0.1 N NaOH solution was taken in a burette and the solution was poured into the milk sample drop by drop and the content of beaker was stirred during that time. Appearance of visible pink colour during titration indicated the end point of the reaction. The volume (ml) of 0.1N NaOH used from burette was noted and the collection was made by using the following formula:

$$\text{Percentage of acidity} = \frac{\text{ml of NaOH used x strength of NaOH x ml eq. wt. lactic acid}}{\text{Weight of Sample}} \times 100$$

Or,

$$\text{Percentage of acidity} = \frac{\text{ml of 0.1 NaOH x 0.09}}{\text{Weight of sample}} \times 100$$

Where 0.09 is the gram of lactic acid equivalent to 0.1 N NaOH. 17.6 ml of milk is equivalent to 18 gms of milk and 0.1N was the normality of NaOH used.

(vi) Determination of pH

P^H was determined by p^H meter-215 (Ciba Corning Diagnostics Ltd. Sudhury, Suffolk, England Co 106 x D) of Dairy Science Laboratory Bangladesh Agricultural University, Mymensingh.

(vii) Determination of protein percentage of milk

The percentage of protein was determined by formal titration method. From the collected milk, sample 10 ml of milk sample was taken in a white porcelain cup by a milk pipette and 10 ml of distilled water was added in the milk sample. Then 0.4 ml of potassium oxalate was added in the milk as a indicator. This well mixed milk sample was kept for 2 minutes without disturbance. Then 0.1 N sodium hydroxide solution (NaOH) was taken in a burette the solution was poured into the milk sample drop by drop and the content of porcelain cup was stirred during the time. Appearance of viable pink colour during titration indicated the end point (T_1) of the reaction and burette reading recorded. After that 2 ml of formalin was added in the mixed milk sample and then 0.1 N sodium hydroxide solution (NaOH) was taken in a burette and this solution was poured into milk sample drop by drop and the content of porcelain cup was stirred during the time and titration was done and again burette reading was obtained (T_2). A blank containing 20 ml distilled water, 0.4 ml saturated solution of potassium oxalate, 1 ml of phenolphthalein and 2 ml of formaldehyde, was titrated against the same NaOH solution. The amount of NaOH solution required was recorded (T_3). The protein content was estimated using by the following formula :

$$\% \text{ of protcin} = \{T_2-(T_1+T_3)\} \times 1.83$$

Where,

T_1 = 1st (initial) burette reading-2nd burette reading

T_2 = 2nd burette reading-Final burette reading

T_3 = NaOH required in balk titration.

(viii) Determination of Ash Percentage of milk

Ten (10) gms of milk sample was taken into a pre-dried and pre-weighed silica-boat. The sample with silica-boat was dried in an oven at a temperature of 138^{0}C for 20 minutes. After that the boat was removed from the oven and was placed in a muffle furnace having a temperature of 450^{0}C for 2 hours. After two hours the silica-boat with sample removed from the muffle farnace and carefully placed into

the desiccator. After cooling the weight of silica-boat with the milk sample taken again and ash remaining was expressed as a percentage of the original weight of the milk sample.

The ash content was estimated by following formula:

$$\text{Percentage of Ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

(ix) Estimation of solids-not-fat and total solids content

The solids-not-fat content and total solids content were estimated using the Babcock formula:

Percentage of solids-not-fat = 0.25L + 0.2F

Total solids % = 0.25L + 1.2F

Where,

L = Correct lactometer reading

And F = Fat percentage of the milk

(x) Estimation of lactose content of milk

The lactose content was estimated by the following formula :

Lactose content of milk = SNF – (Protein + Ash)

Where,

SNF = Solids-not-fat

(xi) **Determination of percent of water added**

$$\text{Percentage of water added} = \frac{\text{Serum solid} - \text{observed serum solid}}{\text{Serum solid}} \times 100$$

(xii) Determination of viable count and coliform count of milk samples.

Agar plates were made according to the procedure described in the standard methods for the examination of dairy products, APHA (1972) . The standard plate count (SPC) agar and violet red agar (VRB) media were used. The experimental procedures followed for the determination of the total viable bacteria in samples and

the detection and enumeration of coliforms were as per recommendation APHA (1972). PCA were incubated at $32^0c \pm 1^0c$ for 48 hours ± 3 hours and VRB plates were incubated at 32^0c for 24 hours.

Standard plate count (SPC) Agar

Formulae:

Peptone Bacto Tryptpham	5.0g
Beef extract	3.0g
Glucose	1g
Agar	15.0g
Total	**24.0g**
Distilled water	1000.00ml

Violet Red Bile (V.R.B.) Agar

Formulae:

Yeast extract	3.0g
Peptone	7.0g
Bile salt	1.5g
Lactose	10.0g
Sodium chloride	5.0g
Neutral red	.03g
Crystal Violet	0.002g
Agar (Bacto agar)	15.0g
Total	**41.5g**
Distilled water	1000.00ml

Source: American Public Heath Association (1972).

APPENDIX TABLE

Appendix Table 1 Analysis of variance table for specific gravity.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant
Between	3	0.000125346	0.0000313365	4.505	**
Within	116	0.001047558	0.000007224537931		
Total	119	0.001172904			

**= Significant at of 1% level

Co-efficient of variation =0.26%

Lsd value =0.0017

Appendix Table 2 Analysis of variance table for Acidity %.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant
Between	3	0.0059194	0.00147985	6.692	**
Within	116	0.0320641	0.00000723		
Total	119	0.0379835			

**= Significant at of 1% level

Co-efficient of variation =10.08%

Lsd valuc– 0.0098

Appendix Table 3 Analysis of variance table for Fat g/kg).

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant	Lsd Value
Between	3	5509.83	1377.46	42.783	**	3.77
Within	116	4668.467	32.196			
Total	119	10178.293				

**= Significant at of 1% level

Co-efficient of variation =13.8%

Appendix Table 4 Analysis of variance table for Solids-not-fat.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant	Lsd Value
Between	3	2457.920	614.480	7.849	**	5.88
Within	116	11352.158	78.291			
Total	119	13810.078				

**= Significant at of 1% level

Co-efficient of variation =11.25%

Appendix Table 5 Analysis of variance table for total solids g/kg.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant	Lsd Value
Between	3	12893.060	3223.265	31.232	**	6.75
Within	116	14964.502	103.203			
Total	119	27857.562				

**= Significant at of 1% level

Co-efficient of variation =8.47%

Appendix Table 6 Analysis of variance table for water g/L.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant	Lsd Value
Between	3	11884.118	2971.030	18.928	**	8.33
Within	116	22759.491	156.962			
Total	119	34643.610				

**= Significant at of 1% level

Co-efficient of variation =1.42%

Appendix Table 7 Analysis of variance table for protein g/kg.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant	Lsd Value
Between	3	10.360	2.590	2.719	*	0.64
Within	116	138.100	0.952			
Total	119	148.460				

*= Significant at of 5% level

Co-efficient of variation =2.78%

Appendix Table 8 Analysis of variance table for lactose g/kg.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant	Lsd Value
Between	3	1628.442	407.110	9.083	**	4.45
Within	116	6498.906	44.820			
Total	119	8127.348				

**= Significant at of 1% level

Co-efficient of variation =18.06%

Appendix Table 9 Analysis of variance table for ash g/kg.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant	Lsd Value
Between	3	0.593	0.148	4.404	**	0.12
Within	116	4.881	0.034			
Total	119	5.474				

**= Significant at of 1% level

Co-efficient of variation =2.68%

Appendix Table 10 Analysis of variance table for percent of water.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant	Lsd Value
Between	3	2224.877	556.219	8.779	**	5.29
Within	116	9186.921	63.358			
Total	119	11411.798				

**= Significant at of 1% level

Co-efficient of variation =110.05%

Appendix Table 11 Analysis of variance table for total viable bacteria.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant	Lsd Value
Between	3	123750.693	30937.673	61.030	**	14.97
Within	116	73504.267	506.926			
Total	119	197252.960				

**= Significant at of 1% level

Co-efficient of variation =36.06%

Appendix Table 12 Analysis of variance table for coliform bacteria /ml.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant	Lsd Value
Between	3	2572.907	643.227	154.043	**	1.35
Within	116	605.467	4.176			
Total	119	3178.373				

**= Significant at of 1% level

Co-efficient of variation =22.08%

Appendix Table 13 Analysis of variance table for specific gravity.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant
Between	3	0.000	0.000	2.839	*
Within	76	0.000	0.000		
Total	79	0.000			

*= Significant at of 5% level

Co-efficient of variation =0.22%

Appendix Table 14 Analysis of variance table for acidity.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant
Between	3	0.000	0.000	2.601	NS
Within	76	0.004	0.000		
Total	79	0.004			

NS = Non Significant

Co-efficient of variation =4.55%

Appendix Table 15 Analysis of variance table for fat.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant	Lsd Value
Between	3	1915.650	638.550	57.616	**	2.21
Within	76	842.300	11.083			
Total	79	2757.950				

**= Significant at of 1% level

Co-efficient of variation =8.59%

Appendix Table 16 Analysis of variance table for solids-not-fat.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant	Lsd Value
Between	3	337.110	112.370	3.137	*	3.02
Within	76	2722.730	35.825			
Total	79	3059.840				

*= Significant at of 5% level

Co-efficient of variation =7.69%

Appendix Table 17 Analysis of variance table for total solids.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant	Lsd Value
Between	3	2822.017	940.672	14.762	**	5.30
Within	76	4842.998	63.724			
Total	79	7665.015				

**= Significant at of 1% level

Co-efficient of variation =6.84%

Appendix Table 18 Analysis of variance table for water.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant	Lsd Value
Between	3	2799.300	933.100	14.657	**	5.30
Within	76	4838.209	63.661			
Total	79	7637.510				

**= Significant at of 1% level

Co-efficient of variation =0.90%

Appendix Table 19 Analysis of variance table for % water added.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant	Lsd Value
Between	3	473.593	157.864	3.620	*	3.34
Within	76	3313.995	43.605			
Total	79	3787.587				

*= Significant at of 5% level

Co-efficient of variation =76.45%

Appendix Table 20 Analysis of variance table for total viable count.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant	Lsd Value
Between	3	695650000	231883333.3	11.110	**	3038.68
Within	76	1586300000	20872368.4			
Total	79	2281950000				

**= Significant at of 1% level

Co-efficient of variation =14.49%

Appendix Table 21 Analysis of variance table for coliform count.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant	Lsd Value
Between	3	1110.00	370.000	0.857	NS	13.81
Within	76	32810.000	431.711			
Total	79	33920.000				

NS= Non Significant

Co-efficient of variation =23.35%

Appendix Table 22 Analysis of variance table for acidity.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant
Replication	4	0.000	0.000	2.4509	
Factor A	8	0.226	0.028	3434.7698	**
Factor B	3	0.020	0.007	820.9449	**
AB	24	0.04	0.002	203.3114	**
Error	140	0.001	0.000		
Total	179	0.288			

**= Significant at 1% level

Co-efficient of variation =1.66%

Appendix Table 23 Analysis of variance table for acidity.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant
Replication	2	0.000	0.000	4.4238	
Factor A	8	0.217	0.027	3735.2954	**
Factor B	5	0.021	0.004	576.3827	**
AB	40	0.024	0.001	82.0323	**
Error	106	0.001	0.000		
Total	161	0.262			

**= Significant at 1% level

Co-efficient of variation =1.47%

Appendix Table 24 Analysis of variance table for acidity.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant
Replication	2	0.000	0.000	0.0261	
Factor A	17	0.126	0.007	834.6354	**
Factor B	5	0.002	0.000	36.2420	**
AB	85	0.001	0.000	1.9269	**
Error	214	0.000	0.000		
Total	323	0.131			

**– Significant at 1% level

Co-efficient of variation =1.87%

Appendix Table 25 Analysis of variance table for Acidity %.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-value	Level of significant
Between	4	0.009572075	0.00239302	2.58	*
Within	75	0.069459125	0.00092613		
Total	79	0.0790312			

**= Significant at of 1% level

Co-efficient of variation =10.08%

Lsd value= 0.0214