EVALUATION OF PHYSICO-CHEMICAL PROPERTIES AND DETECTION OF ADULTERANTS OF THE UHT MILK SAMPLE AVAILABLE AT LOCAL MARKETS IN BANGLADESH

A THESIS
BY

MD. SHIHABUL AWAL
Student No.: 1205043
Session: 2012-13
Semester: January – June, 2013

MASTER OF SCIENCE (M S)
IN
FOOD PROCESSING AND PRESERVATION

DEPARTMENT OF FOOD PROCESSING AND PRESERVATION
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
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In partial fulfillment of the requirements for the degree of

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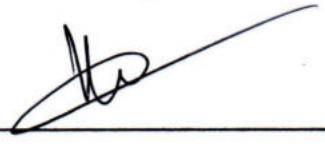

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JUNE, 2013

DEDICATED TO
MY
BELOVED PARENTS

ACKNOWLEDGEMENT

I express the deepest sense of gratefulness to the "Almighty Allah" Who has enable me to complete the thesis work and to prepare this thesis in partial fulfillment of the requirements for the degree of Master of Science in Food Processing and Preservation.

The author expresses his heartfelt gratitude and indebtedness to his reverend supervisor Professor Md. Ruhul Amin, Department of Agricultural and Industrial Engineering, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur for his tremendous help, co-operation, advice and constructive criticism throughout the period of research work as well as in the preparation of the thesis.

The author is highly grateful and indebted to his Co-supervisor, S M Kamrul Hasan, Assistant Professor, Deparment of Food Processing and Preservation, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur for his innovative suggestions, kind co-operation and generous help in completing this study.

The author is extremely glad to take opportunity to express his heartfelt thanks and gratitude to his beloved teachers Dr. Maruf Ahmed, Associate Professor, Deparment of Food Processing and Preservation, Professor Dr. Md. Kamal Uddin Sarker, Department of Agricultural and Industrial Engineering, Professor Dr. Mohammad Shiddiqur Rahman, Department of Agricultural and Industrial Engineering, Md. Sazzat Hossain Sarker, Associate Professor, Deparment of Food Engineering and Technology, and Md. Mojaffor Hosain, Lecturer, Deparment of Food Processing and Preservation, Hajee Mohammad Danesh Science and Technology University, Dinajpur for their kind co-operation during this study period.

A special note of thanks to Md. Raihanul Haque, Lecturer, Department of Food Engineering Technology and all of my friends Md. Imteaj Zubair and Faridul Islam and younger brothers Rony, Azad, Manik, Krishno, Shazedul Awal and Manob for their support. Cordial thanks to the lab attendants of the Department of Food Processing and Preservation and Department of Dairy and Poultry Science for their excellent service during research period.

Finally, I express my deepest gratefulness to my beloved parents and brother for their continuous inspiration, blessings and sacrifices which will never be forgotten.

The Author

ABSTRACT

The study was undertaken to evaluate the UHT milk samples (four brands) available at local markets in Bangladesh. The study was conducted to analyze physiochemical properties of UHT milk together with the determination of adulterants present in it. Results showed that maximum values of protein (3.417%), fat (3.50%) and moisture contents (89.13%) were observed in Farm fresh, Aarong and Farm Fresh, and RD, respectively. Minimum values of protein (3.202%), fat (3.133%) and moisture content (88.64%) were observed in PRAN, RD, and Farm Fresh, respectively. The sample of UHT milk of PRAN showed the highest value for titratable acidity (0.1917%) whereas the sample of RD showed the lowest value (0.1717%). Moreover, the pH for the samples was measured 6.1 to 6.3. The evaluated UHT milk samples were found to have 27.87 to 27.97, 1.027 to 1.0281, 7.742 to 7.865% and 10.88 to 11.36% for CLR (corrected lactometer reading), specific gravity, SNF (Solid not fat) and TS (Total solids), respectively. The clot on boiling and alcohol test for the different UHT milk showed the negative result. The examined UHT milk samples were free from adulterants (except added water) i.e. hydrogen peroxide, rosolic acid, formaldehyde, starch, cane sugar, carbonates, skim milk powder, sodium chloride and pulverized soap. The maximum values of added water in milk samples were observed in RD (3.229%), while minimum in Farm Fresh (1.688%). The statistical analysis showed that Farm Fresh UHT milk was significantly different ($p<0.05$) from PRAN, RD and Aarong milk samples and found to have more acceptable.

CONTENTS

CHAPTER	TITLE	PAGE NO.
	ACKNOWLEDGEMENT	**i**
	ABSTRACT	**ii**
	CONTENTS	**iii**
	LIST OF TABLES	**vi**
	LIST OF FIGURES	**vii**
	LIST OF APPENDICES	**viii**
I	**INTRODUCTION**	**1**
II	**REVIEW OF LITERATURE**	**4**
	2.1 Milk for human consumption	4
	2.2 Production of milk	4
	2.3 Constituents of milk	6
	2.4 Physico-chemical parameters of milk	11
	2.5 Raw milk quality	14
	2.6 Oxidation of milk	16
	2.7 Fluid milk processing	17
	2.8 Milk adulteration	26
III	**MATERIALS AND METHODS**	**28**
	3.1 Collection of samples	28
	3.2 Physico-chemical quality test of the samples	28
	3.2.1 Protein content	28
	3.2.2 Fat content	29
	3.2.3 Titratable acidity content	30
	3.2.4 pH content	30
	3.2.5 Corrected lactometer reading	30
	3.2.6 Specific gravity	31
	3.2.7 Solids not fat	31
	3.2.8 Total solids (TS)	31
	3.2.9 Water content	32
	3.2.10 Clot on boiling test	32
	3.2.11 Alcohol test	32

CONTENTS (contd.)

CHAPTER	TITLE		PAGE NO.
	3.3 Detection of adulterants in milk sample		32
		3.3.1 Added water content	32
		3.3.2 Hydrogen peroxide	33
		3.3.3 Rosolic acid test (soda)	33
		3.3.4 Formaldehyde	33
		3.3.5 Starch	33
		3.3.6 Cane sugar	34
		3.3.7 Carbonates	34
		3.3.8 Skim milk powder	34
		3.3.9 Sodium chloride	34
		3.3.10 Pulverized soap	34
	3.4 Statistical analysis		35
IV	**RESULTS AND DISCUSSION**		**36**
	4.1 Physico-chemical parameters of UHT milk		36
		4.1.1 Protein content	36
		4.1.2 Fat content	37
		4.1.3 pH content	38
		4.1.4 Water content	40
		4.1.5 Solids not fat content	41
		4.1.6 Titratable acidity	41
		4.1.7 Corrected lactometer reading	43
		4.1.8 Specific gravity	43
		4.1.9 Total solids content (TS)	43
		4.1.10 Clot on boiling test	44
		4.1.11 Alcohol test	44
	4.2 Adulteration in milk		45
		4.2.1 Added water content	45
		4.2.2 Hydrogen peroxide	46
		4.2.3 Soda (Rosolic acid)	47

CONTENTS (contd.)

CHAPTER	TITLE		PAGE NO.
	4.2.4	Formaldehyde	47
	4.2.5	Starch	47
	4.2.6	Cane sugar	47
	4.2.7	Carbonates	48
	4.2.8	Skim milk powder	48
	4.2.9	Sodium chloride	48
	4.2.10	Pulverized soap	48
V	**SUMMARY AND CONCLUSION**		**49**
	REFERENCE		**51**
	APPENDICES		**66**

LIST OF TABLES

TABLE	TITLE	PAGE NO.
2.1	Cow milk production in Bangladesh	5
2.2	Production of milk from different animals 2006-2009	5
2.3	Composition of cow and buffalo milk	9
2.4	Mineral and vitamin concentrations in milk	10
2.5	Chemical composition of milk of different breeds of cow	11
4.1	Summary of the results of physico-chemical parameters of UHT milk samples available in local market	42
4.2	The result COB and alcohol for UHT milk	44
4.3	Summary of the result of adulteration test of UHT milk sample	45

LIST OF FIGURES

FIGURE	TITLE	PAGE NO.
4.1	Protein content of different UHT milk	37
4.2	Fat content of different UHT milk	38
4.3	pH content of different UHT milk	39
4.4	Water content of different UHT milk	40
4.5	SNF content of different UHT milk	41
4.6	Added water content of different UHT milk	46

LIST OF APPENDICES

APPENDIX	TITLE	PAGE NO.
1	Pasteurized milk standard (BSTI, 2002)	66
2	Six independent data for protein content (%)	66
3	Analysis of variance (ANOVA) for protein content	66
4	Duncan's multiple range test (DMRT) for protein content	67
5	Six independent data for fat content (%)	67
6	Analysis of variance (ANOVA) for fat content	67
7	Duncan's multiple range test (DMRT) for fat content	68
8	Six independent data for pH content	68
9	Analysis of variance (ANOVA) for pH content	68
10	Duncan's multiple range test (DMRT) for pH content	69
11	Six independent data for water content (%)	69
12	Analysis of variance (ANOVA) for water content	69
13	Duncan's multiple range test (DMRT) for water content	70
14	Six independent data for solids not fat (SNF)	70
15	Analysis of variance (ANOVA) for solids not fat (SNF)	70
16	Duncan's multiple range test (DMRT) for solids not fat (SNF)	71
17	Six independent data for titratable acidity	71
18	Six independent data for corrected lactometer reading (CLR)	71
19	Analysis of variance (ANOVA) for corrected lactometer reading (CLR)	72
20	Six independent data for specific gravity (Sp. Gr.)	72
21	Six independent data for total solids (TS)	72
22	Analysis of variance (ANOVA) for total solids (TS)	73
23	Duncan's multiple range test (DMRT) for total solids (TS)	73
24	Six independent data for percentage of added water content	73
25	Analysis of variance (ANOVA) for percentage of added water content	74
26	Duncan's multiple range test (DMRT) for percentage of added water content	74

CHAPTER 1
INTRODUCTION

CHAPTER I

INTRODUCTION

Milk is very valuable food, readily digested and absorbed. It consists of nutrients, which are needed for proper growth and maintenance of body. Milk and milk products form a significant part of the diet and a substantial amount of our food expenditures goes on milk and other dairy products (El-Loly *et al.*, 2013). Milk is highly susceptible to bacterial contamination due to its excellent nutritional composition and it is easily perishable (Dey and Karim, 2013; OECD, 2005). Furthermore, it can easily be contaminated by many different sources including the udder and body of cows, dust from the air, water supply, and unhygienic conditions during transportation (Hossain *et al.*, 2011; El-Loly *et al.*, 2013). Thus milk and the dairy products can be important sources of food borne pathogens (Oliver *et al.*, 2005) resulting in high health danger eventually causing death of consumers.

On the contrary, consumer prefers wholesome and nutritious food products processed in a sound and sanitary manner thus it is free from pathogens. For fulfilling consumer's demand, production of quality milk is essential. In addition to, quality milk is the milk of normal chemical composition, completely free from harmful bacteria and toxic substances (Khan *et al.*, 2008). For this reason, different heat treatment methods such as pasteurization, sterilization and ultra high temperature (UHT) etc. with integrated aseptic packaging are given to raw milk to remove pathogenic & spoilage causing micro-organisms, and to increase the shelf life of milk (Hassan *et al.*, 2009).

Pasteurization is intended to kill only pathogenic micro-organisms present in milk while sterilization is the term applied to a heat treatment process which has a bactericidal effect greater than pasteurization. However, ultra high temperature (UHT) treatment destroys all microorganisms that are likely to grow under the normal conditions of storage and transportation (Frye and Donnelly, 2005). Although pasteurization of milk effectively removes potential pathogenic bacteria, the heat process is not sufficient to destroy heat-resistant bacterial spores. Further, UHT processing of milk was originally developed with the objective of producing sterile milk i.e. free from all micro-organism since UHT milk has longer shelf life than the other heat treated milk (Mehta, 1980).

Milk is a highly perishable biological material because it is very susceptible to microbial and chemical degradation even stored at refrigeration temperatures. Therefore, it is a major challenge to manufacture sterile milk that will retain good sensory characteristics and physical stability during storage at room temperature for several months. For this reason, UHT processing involves heating milk in a continuous-flow system to a high temperature (135–145°C) and holding it at that temperature for a short time (1–10 seconds) followed by rapid cooling. This produces a "commercially sterile" product, that is, a product in which bacterial growth is highly unlikely to occur under normal storage conditions (25°C). Moreover, this rapid heat transfer rate minimizes undesirable changes in the taste and nutritional quality of the resulting product (Datta and Deeth, 2007).

In Bangladesh, milk is mostly produced through non-standardized methods. In most cases, it is supplied to the consumers by milkmen from rural to urban areas through poor hygienic chains. As a result, there is a chance of introducing germs into the milk further decreasing its quality. So, it is naturally of great importance that such a valuable and highly perishable food should be delivered to the consumer in a wholesome form (Hossain *et al.*, 2011). As we know, UHT milk products are free from micro-organisms and can be delivered in wholesome form to the consumers since ultra high temperature (UHT) produced milk gets a lot of attention in Bangladesh. Moreover, its consumption is increasing day by day due to its hygienic quality and shelf stable at normal storage conditions. Though UHT treated milk free from micro-organism due to its high heat treatment some quality parameters may loss. Hence, the physicochemical properties of the UHT milk samples varied among the manufacturers because the temperature is given to destroy the germs may vary from manufacturer to manufacturer based on quality of raw milk samples.

Additionally, milk is adulterated by the dairy farmers/manufacturer in certain areas of the world where water, starch solutions, industrial alkalis, and nitrite are common materials intentionally added in milk (Mabrook and Petty, 2003) for maintaining the wholesomeness and nutritional quality of milk before/after processing. Furthermore, various preservatives like formalin and some antibiotics are also added in milk to increase its shelf life (Afzal *et al.*, 2011). These chemical substances are called adulterant which should not contain within other substances (e.g. food and beverages) for legal or other reasons. The addition of adulterants is called adulteration (Lakshmi *et al.*, 2012). Normally, the adulteration in food is done either for financial gain or lack of proper hygienic conditions of processing, storing,

transportation and marketing (Lateef *et al.*, 2013). However, milk adulteration leads to economic losses, deterioration of the quality of end products, and a risk to consumers' safety (Mabrook and Petty, 2003). Therefore, it is important for the milk industry to confirm the quality of raw milk supplied by dairy farmers and for consumer agencies to verify the quality of fresh milk purchased from the market.

From the above point of view the research was conducted with the following objectives-

1. To analyze the physicochemical properties of UHT milk available at local market in Bangladesh.
2. To detect the presence of adulterants in the UHT milk samples.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 Milk for Human Consumption

Milk is defined to be the lacteal secretion, practically free from colostrums, obtained by the complete milking of one or more healthy cows, five days after and 15 days before parturition, which contains not less than 8.5 percent milk solids-not-fat and not less than 3.5 percent milk fat (U.S. Public Health Service, 1965; Itzerott, 1960). Milk considered as an attractive source of energy, proteins, and calcium for infants, young children and elderly people who have few alternative sources available for nutrients; even it is a part of daily diets of most of the people (Apurva *et al.*, 2012).

Milk from domestic animals has provided food for humans for more than 8500 years. Milk contains a wide range of readily bio-available nutrients, which enables this nutrient-dense product to be the sole food for neonates and infants during the first stage of growth and development. Moreover, milk and dairy products make a significant contribution to the total supply of nutrients for adolescents and adults. From the early 1900s, public health authorities encouraged the consumption of milk and milk products to improve the nutritional status of the population, especially children (Dairyforall.com/2013).

Milk is an excellent growth medium for bacteria and can easily be contaminated by many different sources including the udder and body of cows, dust from the air, litter, floor, flies, insects and rodents, water supply, hands and clothes of the milker, utensils, bottles, atmosphere etc. (Ensminger *et al.*, 1994; Heineman, 1919; Cousin, 1982). Thus milk and the dairy products can be important sources of food borne pathogens (Oliver *et al.*, 2005).

2.2 Production of Milk

The milk production of last 2011- 2012 fiscal year of Bangladesh was 3.46 million tons against the demand of 13.50 million tons. (Livestock policy and action plan, 2012).

Table 2.1 Cow milk production in Bangladesh

Year	Production(tons)	Year	Production (tons)	Year	Production (tons)
2001	784000	2005	806000	2009	827000
2002	791000	2006	812000	2010	829600
2003	797000	2007	818000	2011	832000
2004	800000	2008	825000	2012	835000

Source: FAO, 2014.

Table 2.2 Production of milk from different animals 2006-2009, 1000 tons

	Sheep milk	Goat milk	Cow milk	Camel milk	Buffalo milk	Milk total
Developed	327	2516	320886	0	228	327000
Formerly centrally planned economics	101	858	99367	0	13	101000
Industrialized	243	1782	238381	0	221	243000
Developing	309	10623	221174	1292	69983	309000
East & Southeast Asia	45	587	39479	19	3124	44500
China	39	265	34950	15	2925	39300
Rest of East & Southeast Asia	5	322	4529	4	199	5207
Latin America & the Caribbean	62	449	61811	0	0	62300
Brazil	20	136	19976	0	0	20100
Rest of Latin America & the Caribbean	42	312	41836	0	0	42200
South Asia	126	5751	55972	0	64520	126000
India	89	2927	43466	0	42799	89200
Rest of south Asia	37	2824	12506	0	21721	37100
Near East & North Africa	35	1231	27924	142	2333	34500
Sub-Saharan Africa	23	2391	18691	1127	0	23300
World	8641	13144	542069	1292	70211	635000

Source: FAOSTAT, 2011.

2.3 Constituents of Milk

The major chemical components of milk include water, fats, proteins, carbohydrates, minerals, organic acids, enzymes and vitamins (Dobrzanski *et. al.*, 2005). Milk and milk products are the most diversified of the natural foodstuffs in terms of composition, contains more than twenty different trace elements (Stawarz *et al.*, 2007). Most of them are essential and very important such as copper, zinc, manganese and iron. These metals are cofactors in many enzymes and play an important role in many physiological functions of man and animals (Koh and Judson, 1986).

The major constituents of milk are: water, fat, protein, lactose, ash or mineral matter. The minor constituents are: phospholipids, sterols, vitamins, enzymes, pigments etc. The true constituents of milk are milk, fat, casein, lactose (Sukumar, 2006).

According to Byron *et al.* (1974), the average composition of milk are (i) Water (87.20%), (ii) Protein (3.50%), (iii) Fat (3.70%), (iv) Milk sugar or lactose (4.90%), (v) ash (0.70%) and (vi) dry matter (12.80%). The constituents may vary with breed, type of feed, stage of lactation, season and age of the cow etc. and also between individuals of the same breed.

Buffalo and cow milk contains 7.6, 4.5 % fat, 3.8, 3.8 % protein, 5.1, 4.9 % lactose, 0.78, 0.72 % ash and 17.0, 13.9 % total solids, respectively (Khan *et al.*, 2005). Compositions of cow and buffalo milk are shown in table 2.1.

2.3.1 Water

Water is the medium in which all other components of milk are dissolved or suspended. Water content varies from 83.18 to 87.3% in milk of different species. A small percentage of water in milk is hydrated to lactose and salts, while some portion is bound with proteins (Aneja *et al.*, 2002).

2.3.2 Fat

Normally, fat (or lipid) makes up from 3.5 to 6.0% of milk, varying between breeds of cattle and with feeding practices. A ration too rich in concentrates that do not elicit rumination in the cow may result in milk with a depressed percentage of fat (2.0 to 2.5%). The majority of milk fat is in the form of triglycerides formed by the linking of glycerol and fatty acids. The proportions of fatty acids of different lengths determine the melting point of fat and thus the

consistency of the butter derived from it. Milk fat contains predominantly short-chain fatty acids (chains of less than eight carbon atoms) built from acetic acid units derived from fermentation in the rumen. This is a unique feature of milk fat compared with other kinds of animal and plant fats. The long chain fatty acids in milk are primarily the unsaturated (hydrogen deficient) acids, with the predominant one being oleic (18-carbon chain), and polyunsaturated linoleic and linolenic acids (http://babcock.wisc.edu/node/198).

Milk is an emulsion or colloid of butterfat globules within a water-based fluid. Each fat globule is surrounded by a membrane consisting of phospholipids and proteins; these emulsifiers keep the individual globules from joining together into noticeable grains of fat and also protect the globules from the fat-digesting activity of enzymes found in the fluid portion of the milk. The fat-soluble vitamins A, D, E and K are found within the milk fat portion of the milk (Harold, 1984). The average size of fat globules in buffalo milk is larger (4.15 to 4.60 μm) than that of cow milk (3.36 to 4.15 μm).

2.3.3 Proteins

Most of the nitrogen in the milk is found in the form of protein. The building blocks of all proteins are the amino acids. There are 20 amino acids that are commonly found in proteins. The order of the amino acids in a protein, which is determined by the genetic code, gives the protein a unique conformation. In turn, the spatial conformation of the protein gives it a specific function. The concentration of protein in milk varies from 3.0 to 4.0% (30-40 grams per liter). The percentage varies with the breed of the cow and in proportion to the amount of fat in the milk. There is a close relationship between the amount of fat and the amount of protein in milk the higher the fat, the higher the protein. The protein falls into two major groups: caseins (80%) and whey proteins (20%). Historically, this classification followed the process of cheese making, which consists of separating the casein curd from the whey after the milk has clotted under the action of rennin or rennet (a digestive enzyme collected from the stomach of calves). The behavior of the different types of caseins (α, β and κ) in milk when treated with heat, different pH (acidity) and different salt concentrations provide the characteristics of cheeses, fermented milk products and different forms of milk i.e. condensed, dried, etc. Occasionally, infants or young children are allergic to milk because their bodies develop a reaction to the proteins in the milk. The allergy causes rash, asthma, and/or gastrointestinal disorders (colic, diarrhea, etc.). In cases of allergies, goat milk is

often used as a substitute; however, sometimes hydrolyzed casein milks must be used (http://babcock.wisc.edu/node/198).

The largest structures in the fluid portion of the milk are casein protein micelles: aggregates of several thousand protein molecules, bonded with the help of nanometer-scale particles of calcium phosphate. Each micelle is roughly spherical and about a tenth of a micrometer across. There are four different types of casein proteins, and collectively they make up around 80% of the protein in milk, by weight. Most of the casein proteins are bound into the micelles. There are several competing theories regarding the precise structure of the micelles, but they share one important feature: the outermost layer consists of strands of one type of protein, k-casein, reaching out from the body of the micelle into the surrounding fluid. These kappa-casein molecules all have a negative electrical charge and therefore repel each other, keeping the micelles separated under normal conditions and in a stable colloidal suspension in the water-based surrounding fluid (Fox and McSweeney, 1998). Caseins are heat stable. Heat has little or no effect on casein molecules since they exist naturally in an open and extended state at higher temperatures.

Pandya and Haenlin (2009) have reported that as compared to cow milk, buffalo milk is richer in total proteins, particularly casein and whey proteins. However, the proportion of various protein fractions is similar in milk of both species. Distinct differences exist in the physico-chemical makeup of casein in buffalo and cow milk. The buffalo casein micelle is more opaque, about three times, when suspended in a different medium, than cow milk micelle. Buffalo casein has superior whitening as compared to cow casein due to a higher proportion of calcium present in buffalo milk.

According to Walstra *et al.* (1999) milk contains dozens of other types of proteins beside the caseins. They are more water-soluble than the caseins and do not form larger structures. Because these proteins remain suspended in the whey left behind when the caseins coagulate into curds, they are collectively known as whey proteins. Whey proteins make up approximately twenty percent of the protein in milk, by weight. β -Lactoglobulin, α - lactalbumin and proteose-peptone are the most common whey protein by a large margin.

Both the fat globules and the smaller casein micelles, which are just large enough to deflect light, contribute to the opaque white color of milk. Sukumar (1980) described that the fat globules contain some yellow-orange carotene, enough in some cattle breeds, such as, Guernsey and Jersey cattle, to impart a golden or "creamy" hue to a glass of milk. The

riboflavin in the whey portion of milk has a greenish color, which sometimes can be discerned in skimmed milk or whey products. Harold (1984) suggested that fat-free skimmed milk has only the casein micelles to scatter light, and they tend to scatter shorter-wavelength blue light more than they do red, giving skimmed milk a bluish tint.

Table 2.3 Composition of Cow and Buffalo milk

Constituent (%)	Cow milk	Buffalo milk
Water	86.50	83.18
Fat	4.39	6.71
Protein	3.30	4.52
Lactose	4.44	4.45
Total solids	13.50	16.82
Solids not fat	9.11	10.11
Ash	0.73	0.80
Calcium	0.12	0.18
Magnesium	0.01	0.02
Sodium	0.05	0.04
Potassium	0.15	0.11
Phosphorous	0.10	0.10
Citrate	0.18	0.18
Chloride	0.10	0.07

Source: Aneja *et al.* (2002)

2.3.4 Carbohydrates

Lactose, also called as milk sugar, is the major carbohydrate of milk. The carbohydrate lactose gives milk its sweet taste and contributes approximately 40% of whole cow's milk's calories. Lactose is a disaccharide composite of two simple sugars, glucose and galactose. Buffalo milk usually contains lactose in the range of 4.7-5.0%, while cow milk has slightly lower amount of lactose in the range of 4.5-4.8%. Lactose has only 16-33% of the sweetening power of sucrose. Varman and Sutherland (2001) have explained that lactose makes a major contribution to the colligative properties of milk, such as, osmotic pressure, freezing point depression and boiling point elevation. Lactose finds use as food ingredient due to protein stabilizing properties and low relative sweetness. Lactose may also be used as

partial replacer of sucrose in icing and toppings to improve mouth-feel without excess sweetness. It is also added to bakery products such as biscuits to impart controlled degree of Millard browning, a reaction considered undesirable in many foods. Lactose is a significant source of dietary energy and may promote calcium absorption (www.lactose.com).

2.3.5 Minerals and Vitamins

Milk is an excellent source of most minerals required for the growth of the young. The digestibility of calcium and phosphorus are unusually high, in part because they are found in association with the casein of the milk. As a result, milk is the best source of calcium for skeletal growth in the young and maintenance of bone integrity in adults. Another mineral of interest in the milk is iron. The low iron concentration in milk cannot meet the needs of the young, but this low level turns out to have a positive aspect because it limits bacterial growth in Milk-iron is essential for the growth of many bacteria (http://babcock.wisc.edu/node/198).

Table 2.4 Mineral and Vitamin concentrations in milk

Minerals	(mg/100ml)	Vitamins	µg/100ml
Potassium	138	Vitamin A	30
Calcium	125	Vitamin D	0.06
Chloride	103	Vitamin E	88.0
Phosphorus	96	Vitamin K	17.0
Sodium	58	Vitamin B_1	37.0
Sulfur	30	Vitamin B_2	180.0
Magnesium	12	Vitamin B_6	46.0
Trace minerals[1]	<0.1	Vitamin B_{12}	0.42
[1]Includes cobalt, copper, iron, manganese, molybdenum, zinc, selenium, iodine and others.		Vitamin C	1.7

Source: http://babcock.wisc.edu/node/198

Table 2.5 Chemical composition of milk of different breeds of cows

Cow species	Percent of Composition				
	water	Fat	Protein	Lactose	Ash
Holstein	87.74	3.40	3.22	4.87	0.68
Shorthorn	87.19	3.94	3.32	4.99	0.70
Ayreshire	87.10	4.00	3.58	4.67	0.68
Brown swiss	86.59	4.01	3.61	5.04	0.73
Guernsey	85.39	4.95	3.91	4.93	0.74
Jersey	85.09	5.37	3.92	4.93	0.71
Sindhi	86.07	4.90	3.42	4.91	0.70
Gir	86.44	4.73	3.32	4.85	0.66
Tharparssar	86.58	4.55	3.36	4.83	0.68
Sahiwal	86.42	4.55	3.33	5.04	0.66

Source: Dairyforall, 2013.

2.4 Physico-Chemical Parameters of Milk

2.4.1 Water

Hossain *et al.* (2011), found the water content in UHT milk which was 88.0% to 89.0%, similar to that reported by Hossain *et al.* (2010). The water content of raw milk from dairy farm, dairy shops and street vendor was high, which was 87.60%, 89.70% and 90.50%, respectively (Ibrahim *et al.*, 2012). Hossain *et al.* (2011), observed that the water content with high range (89.0 to 91.0%) of different raw and pasteurized milk. Naveenraj *et al.* (2013) reported that the water content of different raw milk was 82.8% to 84.8%. The water content of raw milk within the standard value (86.8%) was observed by Imran *et al.* (2008).

2.4.2 Fat

Apurva *et al.* (2012), was found the maximum value of fat 5.92% and minimum 5.01% of cow milk which was treated over 100^0C. Hossain *et al.* (2011) found that the fat content of UHT milk, one of the UHT-processed milks, was even less (3.09%) and another high (3.62%); also found maximum (3.75%) and minimum (3.12%) for the raw milk. The fat

content of different Pasteurized milk obtained from Milk vita, Tatka, Farm Fresh, Aarong and RD were 3.51%, 3.06%, 3.26%, 3.41% and 3.33%, respectively (Saha and Ara, 2012). Ibrahim *et al.* (2012) was found that fat content of raw milk samples had an average of 3.6%, while lower results were recorded by Kamel (2000). Hassan *et al.* (2009) was reported that the higher result of fat content of different UHT milk (3.50% to 3.80%). Awan *et al.* (2013) was found the fat content (3.0%) of UHT milk sample.

2.4.3 Protein

Hossain *et al.* (2011) was reported that the higher protein content 3.43%, 3.52% and 3.68% in different UHT milk samples. The protein content of different Pasteurized milk samples obtained from Milk vita, Tatka, Farm Fresh, Aarong and RD were 4.07%, 4.14%, 4.14%, 4.03% and 4.10%, respectively (Saha and Ara, 2012). The protein content of raw milk of two districts of East Wolloga was 3.30% and 3.32% (Tola *et al.*, 2007). Hassan *et al.* (2009) was observed the maximum value of protein 3.70% and minimum 3.30% for different UHT milk. Hossain *et al.* (2010) reported that the protein content was 3.68% and 3.43% for UHT milk samples.

2.4.4 Titratable Acidity

Titratable acidity is a measure of freshness and bacterial activity in milk. Popescu and Angel (2009) reported that high quality milk essentially needs to have less than 0.14 percent acidity. The acidity of the raw milk samples varied largely from one sample to another during the storage period. BSTI (2002) allows a maximum acidity of 0.15% for the pasteurized milks. Hassan *et al.* (2009) found that the titratable acidity was increased during storage of UHT milk; the increased in acidity was 0.11% to 0.18% (first week to 12^{th} week), respectively.

Titratable acidity of milk has long been recognized and employed as an indicator of quality (Griffiths *et al.*, 1988). It is expressed in terms of percentage lactic acid since lactic acid is the principal acid produced by fermentation after milk is drawn from the udder. Fresh milk, however, does not contain any appreciable amount of lactic acid and therefore an increase in acidity is a rough measure of its age and bacterial activity (O'Mahony, 1988). Within a short time after milking, the acidity increases perceptibly due to bacterial activity. The degree of bacterial contamination and the temperature at which the milk is kept are the chief factors influencing acid formation. Therefore, the amount of acid depends on the cleanliness

of production and the temperature at which milk is kept. For this reason, determination of acid in milk is an important factor in judging milk quality. Acidity affects taste as well. When it reaches about 0.3%, the sour taste of milk becomes sensible. At 0.4% acidity, milk is clearly sour, and at 0.6% it precipitates at normal temperature. At acidity over 0.9%, it moulds (Torkar and Teger, 2008).

2.4.5 pH

Hassan *et al.* (2009) found that the pH was decreased during storage of UHT milk; the decreased in pH was 6.81 to 6.20 (first week to 12th week), respectively. The decreased in pH content also observed by Akhtar *et al.* (2003) and the pH content was 6.74 to 6.54 during storage of UHT milk for zero to 90 days. Awan *et al.* (2013) found the pH content was 6.00 of different UHT milk samples. The pH content of raw milk (6.76) was observed by Imran *et al.* (2008).

2.4.6 Solids Not Fat (SNF)

Milk is valued commercially for its two important parameters, the Milk Fats (F) and the Solids-Not-Fat (SNF). The SNF largely consists of proteins, lactose and minerals. These solids are also referred to as 'serum solids'. These two parameters usually form the basis for the basis of payment to milk producers in our country. The term 'Total Solids' (TS) refers to the quantity of SNF plus fat present in milk. It may range from 12 to 17% depending on its source. Milk of different animal species differs widely in composition. All milks contain the same kind of constituents, but in varying amounts. Factors such as the type of protein; the proportion of protein, fat, and lactose; the levels of various vitamins and minerals; and the size of the butterfat globules, and the strength of the curd are among those that may vary. Aneja *et al.* (2002) has reported the average composition of cow and buffalo milk, which is summarized in Table 2.1. Apurva *et al.* (2012), was found the maximum value of SNF 8.45% and minimum 8.21% of cow milk which was treated over 100^{0}C. Hossain *et al.* (2011) was reported that the higher SNF content 8.38%, 8.56% and lower 7.91% in different UHT milk samples. Hossain and Dev (2013) found the SNF content of raw milk was 7.81%.

2.4.7 Specific Gravity

The specific gravity of raw milk from dairy farm, dairy shops and street vendor was 1.032, 1.027 and 1.024, respectively (Ibrahim *et al.*, 2012). Khan *et al.* (2008) observed that the highest specific gravity of raw milk from different farm was 1.0295 and lowest 1.0237.

2.4.8 Total Solids (TS)

Hossain *et al.* (2011) was reported that the higher TS content 12% and lower 11% in different UHT milk samples. The TS of different Pasteurized milk samples obtained from Milk vita, Tatka, Farm Fresh, Aarong and RD were 11.46%, 11.58%, 411.41%, 11.53% and 11.35%, respectively (Saha and Ara, 2012). The TS of raw milk of two districts of East Wolloga was 14.69% and 13.94% observed by Tola *et al.* (2007). The TS of raw milk from dairy farm, dairy shops and street vendor was 12.40%, 10.30% and 9.50%, respectively (Ibrahim *et al.*, 2012).

2.5 Raw Milk Quality

As with all food products, the quality of the raw milk directly affects the quality of the finished, pasteurized product (Cromie, 1992; Hayes, 2001; Nornberg, 2010). Contamination of raw milk can come from several sources including mastitic cows, dirty udders or teats, and poorly cleaned milking and storage equipment (Huck *et al.*, 2007; Huck *et al.*, 2008). Many different bacteria can be found in raw milk including both gram-negative bacteria (e.g. *Pseudomonas spp.*, *Aeromonas, Serratia spp.*, *Achromobacter spp.*, *Alcaligenes, Chromobacterium spp.*, and *Flavobacterium spp.*) and gram-positive bacteria (e.g. *Bacillus spp.*, *Clostridium spp.*, *Paenibacillus spp.*, *Streptococcus spp., Staphylococcus spp.*) (Ternstrom *et al.*, 1993; Surhaug and Stepaniak, 1997; Hayes *et al.*, 2001; Nörnberg, 2010). Psychrotrophic bacteria, which are able to propagate and metabolize at refrigeration temperatures (0-7^{0}C) are major contaminants in raw milk and can be a common cause of fluid milk spoilage (Ternstrom *et al.*, 1993). Psychrotrophic bacteria have become an increasing problem since the introduction of refrigerated raw milk-storage tanks on the dairy farm (Surhaug and Stepaniak, 1997). After the introduction of refrigerated raw milk storage, the rate of raw milk collection from the farm decreased to two or three collections per week. In some cases the milk is being further stored at the processing plant over weekends (Cromie, 1992). Despite the optimum growth temperature of psychrotrophic bacteria, which varies from 15-30^{0}C (Morita, 1975), storage times of two days at 7^{0}C or less, on the farm

allows for these bacteria to grow and eventually dominate the raw milk flora (Cromie, 1992; Surhaug and Stepaniak, 1997). The law requires that the raw milk be stored for no longer than 48 hours on the farm (FDA, 2009).

Mastitis is of major concern when striving for great quality milk. Mastitis is characterized by a bacterial infection of the teat or utter of the cow. When a cow with mastitis is milked it can secrete viable bacterial cells in concentrations of up to 10^7cfu/ml (National Mastitis Council, 2011). There are several bacteria that can cause a mastitis infection including, *Streptococcus, Staphylococcus, Enterococcus* and coliforms such as *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter aerogenes* (National Mastitis Council, 2011). The other major problem associated with mastitis is an increased somatic cell count (SCC). Somatic cells produce heat stable proteases which can break down milk protein and cause off-flavors to develop in the pasteurized product (Ma *et al.*, 2000). This increased proteolysis can also be detrimental to yields of other products such as cheese and yogurt (Politis, 1988). Because of this, the cheese industry offers premium prices for low SCC milk. The fluid milk industry has yet to offer such benefits. There is little research on the effects of increased SCC on fluid milk quality. Ma *et al.* (2000) conducted a study on the effects of SCC on fluid milk quality. The research showed that milk with higher SCC counts had increased proteolytic and lipolytic activity in raw milk. They also found that off-flavors such as bitterness and rancidity in the pasteurized milk were associated with the high levels of proteolysis and lipolysis. Ma *et al.* (2000) found that milk with low SCC retained high organoleptic quality throughout a 21 day shelf life while milk with high SCC developed rancid and bitter off-flavors after 14 days of shelf life. Their study suggests that the onset of rancid and bitter off-flavors in the pasteurized milk is a result of the lipolytic and proteolytic activity in the raw milk. They also suggest that quality premium payment programs based on SCC be initiated to help improve fluid milk quality.

Psychrotrophs are capable of producing both proteolytic and lipolytic enzymes during growth at psychrotrophic temperatures, which may be heat-stable and remain active after pasteurization (Cromie, 1992; Ezzati *et al.*, 2010; Nörnberg, 2010). Proteases produced by psychrotrophic bacteria are capable of spoiling milk after pasteurization. Nörnberg *et al.* (2010) found that the addition of proteases isolated from psychrotrophic bacteria found in raw milk, to UHT milk caused extensive coagulation after only 5 days. These proteases can also cause an increase concentration of peptides in milk, which can cause bitter flavors to predominate (Surhaug and Stepaniak, 1997; Nörnberg, 2010). Lipolytic enzymes that are

produced by some Pseudomonas spp. can hydrolyze triglycerides, which are the main lipid component of milk, creating short chain fatty acids or free fatty acids (FFA). An increase in FFA has been shown to cause strong, offensive defects such as rancidity and bitter off-flavors (Surhaug and Stepaniak, 1997; Nörnberg, 2010).

Non-microbial lipolysis due to LPL can be divided into two overlying categories: spontaneous lipolysis and induced lipolysis. Induced lipolysis is defined as lipolysis that is promoted by shearing of the milk fat globule by physical abuse of the milk, which exposes the lipid substrate to the LPL enzyme. Induced lipolysis can be triggered on the farm, during transportation or in the processing plant (Deeth and Fitz-Gerald, 1995).

Lipoprotein lipase is not heat stable, but the off-flavors caused by its activity in raw milk can be transmitted into the pasteurized product. Because of the heat-instability of LPL, occurrence of off-flavors in the pasteurized milk due to LPL action can be avoided by taking special care during the production, transportation and pre-pasteurization processing steps. One major cause of LPL spoilage in pasteurized milk is the mixture of raw and pasteurized product during processing (Chandan *et al.*, 2008). LPL action can also be promoted pre-pasteurization by different feed types, physical abuse, homogenization, and freezing and thawing of the raw milk. Off-flavors due to lipolytic activity can occur within 24 hours depending on temperature of milk and the degree of activity of the lipase (Clark *et al.*, 2009). Rancidity due to LPL action is most common in un-homogenized milk but can occur in homogenized milk if pasteurization does not immediately follow (Clark *et al.*, 2009). Homogenized raw milk has more surface area that is exposed to lipase and therefore is susceptible to hydrolytic rancidity (Deeth, 1986). The other major spoilage mechanism that occurs pre-pasteurization is oxidation. Vitamin A, milk protein, and lipids are all prone to oxidation which can create off-flavors that carry through to the pasteurized product (Barrefors *et al.*, 1995).

2.6 Oxidation of Milk

There are two different types of oxidation that occur in fluid milk: metal induced-oxidation; and light-induced oxidation. The metal-induced oxidation is primarily associated with unsaturated lipids and commonly occurs due to three common causes: direct contact with raw metals; introducing trace metals to the feed of milking cows; or, the presence of divalent cations (Cu, Fe, Mn) in the water supply used for cleaning milking equipment. Exposure to

these metals catalyses an auto-oxidation of the lipids, forming a free radical, which produces aldehydes, ketones and other compounds that can negatively affect the sensory profile of the raw milk.

Light induced oxidation can be characterized by flavors such as wet-cardboard, medicinal, burnt protein, or chemical like (Clark *et al.*, 2009). Exposure to ultra-violet or fluorescent light triggers two different types of reactions: a vitamin A oxidation; and a protein breakdown reaction. Exposure to sunlight for as little as 15 minutes minutes has been found to cause light induced off-flavors (Chapman, 2006). For the protein breakdown to occur riboflavin is required. Riboflavin is known to be naturally abundant in raw milk (Dunkley *et al.*, 1962).

The quality of the raw material used in UHT processing is of utmost importance. It is arguably more important than for pasteurized products because of the long periods of storage of UHT products at ambient temperature when even very slow development of defects may leads to a defective product. In practice, some manufacturers select milk of the highest quality to use in UHT process in order to minimize processing difficulties and the incidence of storage-related defects. The UHT process destroys all vegetative bacteria and most sporeformers but does not in-activate some of the enzymes produced by psychrotrophic bacteria such as Pseudomonas species, the most common bacterial contaminants of raw milk. Such enzymes are typically produced when the bacterial count exceeds ~10^6 per ml. If milk with such a bacterial count is UHT processed, these enzymes, particularly proteinases and lipases, can remain active in the UHT milk. Since UHT milk is usually kept at room temperature and may be stored for several months, even traces of these enzymes can produce noticeable changes and result in bitter flavor and gelation (from proteinases) and rancid flavors (from lipases) (Nivedita and Hilton, 2007).

2.7 Fluid Milk Processing

Current fluid milk processing practices can be divided into seven general steps, from the farm to the packaged product. These steps include: bulk milk handling and storage, separation, standardization, homogenization, pasteurization, cooling and packaging and storage. Each one of these steps can be broken down into several stages. Bulk milk handling and storage starts on the farm where the cows are milked and the raw milk is cooled over several hours to below 7.2°C and stored in a raw milk bulk tank until pick-up. The milk is

then transported in a tanker truck from the farm to processing plant. In some cases the farm is on the same grounds as the processing plant and no transportation is required. At the processing plant the milk is stored in silos and often mixed with raw milk from other dairy farms. Separation is the first step that takes place in the processing plant. During this step the milk is separated into a heavier skimmed milk fraction and a lighter cream fraction (Chandan *et al.*, 2008). The separation step allows for standardization, which is the next step in the process. During standardization the processor can obtain a predetermined fat content. Blending the cream and skimmed milk fractions allows for the production of products with varying fat contents. Reduced fat milks are fortified with vitamins A and D. Vitamins A and D are both fat soluble so they are removed in the cream fraction and must be added back to the skimmed fraction. This usually takes place between the separation and homogenization step. If the vitamins are added before homogenization it allows for them to be dispersed properly. Homogenization can take place in either one or two stages and is always carried out at temperature of 37.2°C or higher. Often the cream is homogenized after separation and then added back into the skimmed milk during standardization. During the homogenization step the fat globules of the milk are reduced in size and the total surface area of exposed lipids is increased.

2.7.1 Homogenized

According to the United States Public Health Service, homogenized milk is milk which has been treated in such a manner as to insure breakup of the fat globules to such an extent that after 48 hours quiescent storage no visible cream separation occurs on the milk; and the fat percentage of the milk in the top 100 ml of milk in a quart bottle, or of proportionate volumes in containers of other sizes, does not differ by more than 10 per cent of itself from the fat percentage of the remaining milk as determined after thorough mixing. Homogenization refers to the process of forcing the milk through a homogenizer with the object of sub-dividing the fat globules.

2.7.2 Pasteurization

Pasteurization is a mild heat treatment process that destroys a selected group or groups of microorganisms, and then relies on further preservation hurdles to ensure the surviving microorganisms do not grow during storage of that food. Milk is the most widely consumed pasteurized food and the process was first introduced commercially during the 1930s, when

treatments of the order of 63 °C for 30 min were used. Modern milk pasteurization uses an equivalent process of 72°C for 15s (in the UK). Pasteurization is nowadays used extensively in the production of many different types of food, such as fruit products, pickled vegetables, jams and ready meals (Campden BRI, 1992 & 2006). Pasteurization is done to render milk safe for human consumption by destruction of cent per cent pathogenic micro-organisms and to improve the quality of milk by destruction of almost all spoilage organisms (85 to 99 per cent).

2.7.3 Ultra High Temperature (UHT) Processing

UHT is the abbreviation for Ultra High Temperature. UHT heat treatment is a technique for preserving liquid food products by exposing them to brief but intense heating. It is a continuous form of heat processing and it employs intense form of heat treatment where the product is usually packaged aseptically after heating and cooling without exposure to environment. Aseptic filling to avoid re-infection of the product is an integral part of the process. Naturally, this process increases the shelf life of milk and dairy products, sometimes up to 3 months of duration even when stored at room temperature.

The temperature time combination used in this process is usually between 135-150°C for a fraction of second. The advantage of this method is less nutrients destruction because of brief exposure to heat and destruction of all pathogenic and spoilage causing microorganisms, thereby ensuring the safety of the products.

The following are the two methods of UHT treatments that are commonly used.

- Indirect heating and cooling in heat exchangers
- Direct heating by steam injection or infusion of milk into steam and cooling by expansion under vacuum.

Homogenization prevents the milk fat from separating during storage. Once the milk is standardized it is heat-treated. Several pasteurization methods are commonly used for fluid milk processing, specifically HTST and ultra-high temperature (UHT) pasteurization. Low temperature long time (LTLT) pasteurization is not commonly used in fluid milk production since the rise of HTST and UHT pasteurization methods. HTST and UHT pasteurization utilize a regeneration step which makes them more efficient than LTLT pasteurization. Although UHT pasteurization produces a longer shelf life product when packaged

aseptically, there still tends to be a preference for HTST milk by consumers. There are off-flavors associated with the UHT pasteurization process, the most common of which is a cooked flavor (Wustenberg, 2012).

The growth of UHT milk has been remarkable, increasing worldwide in the past 20 years especially in Europe, Asia, and South America. Surprisingly, shelf-stable milk consumption in the U.S. is very low compared to other regions in the world (Burton 1988; Kissell, 2004). UHT processed fluid milk is very popular in other parts of the world; however, the U.S. population has been slow to accept it because of the "cooked" flavor in the UHT milk, their familiarity with fresh milk (Dairy Biz Archive, 2000), and the higher cost of UHT milk (Kissell, 2004).

A number of studies have determined sensory properties of various milk samples including plain milk (Claassen and Lawless, 1992; Frost *et al.*, 2001; Francis *et al.*, 2005), chocolate milk (Thompson *et al.*, 2004), powdered milk (Kamath *et al.*, 1999; Drake *et al.*, 2003) and processed milks that are not specific to UHT milk (Chapman *et al.*, 2001; Fromm and Boor 2004; Clare *et al.*, 2005). In addition, lexicons for milk alternatives, such as soymilk, have been published (Torres-Penaranda and Reitmeier, 2001; Day *et al.*, 2004; Chambers *et al.*, 2006; Keast and Lau, 2006).

2.7.4 Effect of UHT Processing of Milk

Extensive research has reported the presence and characteristics of heat-resistant enzymes in milk and their effects on UHT products during storage. Proteases and lipases are of greatest concern. Although phosphatase activity is always zero after milk has been sterilized, it may be reactivated after prolonged storage, where the extent of reactivation increases with storage time and temperature (Robertson, 2006).

Age gelation is an irreversible phenomenon that occurs during storage of UHT-processed milk products, ultimately transforming the product into a gel. It is considered the most important index of failure associated with this type of product, because once the product has gelled, it has reached the end of its shelf life. The severity of the heat treatment, both prior to and during the sterilization process, critically affects age gelation in UHT milk products, with gelation being less critical in UHT milk than in UHT concentrated milk. Sterilized milk produced by the direct-heat UHT process is more prone to gelation than that prepared using

the indirect method, probably owing to better control over the severity of the heat treatment given in the latter (Rosenberg, 2002).

Researchers are still not sure whether gelation is attributable to enzymic action or chemical and physical processes. For many years, it was considered that coagulation was caused by the slow action of heat-resistant proteases from psychrotrophs such as Pseudomonas spp. However, age gelation has occurred where proteolytic activity was not evident and has not occurred on other occasions when proteolytic activity was evident. A mechanism consisting of an enzymic triggering stage followed by a nonenzymic aggregation phase has been suggested. Although proteolysis is involved, nonenzymic mechanisms play a major role in governing the phenomenon of age gelation, especially those affecting interactions between caseins and whey proteins. The best way of avoiding age gelation is to prevent the development of heat-resistant enzymes in the milk before processing. This can be achieved by preventing contamination by the causal micro-organisms, and particularly by keeping the storage time short and the storage temperature low (e.g., <58°C) to prevent the growth of psychrotrophs (Burton, 1988).

The sorption of dairy flavor compounds (aldehydes and methyl ketones) by LDPE and PP films has been investigated quantitatively in an attempt to assist aseptic processors select appropriate packaging materials for maximum flavor stability. PP sorbed these compounds to a greater extent than LDPE. Headspace analysis of UHT-processed milk packaged in aseptic paperboard cartons revealed a loss of higher molecular weight flavor compounds after 12 weeks' storage, owing to the interaction between the LDPE packaging material and the milk (Hansen and Arora, 1990).

Heat treatment of milk results in denaturation of the whey proteins. The extent of denaturation varies according to the severity of heat treatment, from partial during pasteurization to total during in-bottle sterilization. Loss of available lysine is approximately 1–2% in pasteurized milk and 4–5.5% in UHT milk (Varnam and Sutherland, 1996). The denaturation of whey proteins plays a key role in the development of cooked milk flavor. This is insignificant in pasteurized milk but detectable in UP milk and more extensive in UHT-treated milk, in which cooked flavor is a serious quality defect. Nonenzymic browning reactions that result in darkening of milk color are not readily detectable in UP milk but are more intense in UHT milk. Heat also affects ascorbic acid content: in pasteurized milk 10–25% is lost, and in UP and UHT milk more than 25% is lost. Light-induced losses of

vitamins are also very important in UP and UHT milk. Given that these types of products will normally have a shelf life of more than 30 days, it is imperative that packaging materials impermeable to light be used. Loss of riboflavin can be extensive, followed by loss of vitamin A.

Heating of milk accounts two main problems, age gelation and off flavor development, which limits shelf life of milk. UHT treatment of milk leads to a much larger production of small sized casein micelles compared to raw or pasteurized milk (Singh, 1993). Biochemical processes involve terial proteases and survival of bacterial spores (Singh, 1993; Manji *et al.*, 1988). Proteolysis of UHT milk during storage at room temperature is a major factor limiting the shelf life through changes in its flavor and texture (Datta *et al.*, 2002). The changes ultimately reduce the quality and limit the shelf life of UHT milk via development of off flavors, fat separation and sedimentation, which principally falls into 2 categories, liberation of volatile fatty acids such as butyric acid and oxidation of free or unsaturated fatty acids (Datta *et al.*, 2002).

Above 135°C the protein deposited on the fat globule membrane form a network which makes the membrane denser and less permeable (Fink and Kessler, 1986). There is an increase in acidity and viscosity with a decrease in pH with the storage time increased both in UHT. Clare *et al.* (2005) determined that sweet aromatic flavor and sweet taste of UHT milk decreases during storage.

2.7.5 Effect on Fouling

UHT processing often causes milk solids to attach tenaciously to the heat exchanger surface, a phenomenon known as fouling or burn-on. It is a major concern during UHT processing. Several factors affect the rate of fouling, one of which is the quality of the raw milk. Some manufacturers use an alcohol stability test on the raw milk as a guide to its propensity to foul during heat processing. For good stability, the raw milk should be stable in at least 74% alcohol (IDF 1981). The pH has a major effect on both the alcohol stability and the rate of fouling; the former decreases and the latter increases as the P^H of the milk decreases. This may be due, at least in part, to an increase in ionic calcium that is known to be related to fouling; the higher the ionic calcium, the greater the propensity for fouling. Goats' milk has higher ionic calcium than cows' milk and a much greater tendency to fouling (Nivedita and Hilton, 2007).

A decrease in pH often accompanies bacterial growth in raw milk so milk with a high bacterial count may foul more during processing. High bacterial counts in raw milk occur after prolonged storage or storage at an elevated temperature. Kastanas *et al.* (1995) found that good quality raw cows' milk could be stored for at least 7 days at 2°C prior to UHT processing at 140°C before unacceptable fouling was observed.

Seasonal variation in the composition of milk also affects the rate of fouling during UHT processing. Grandison (1988) observed a twofold range in processing run times over a 12-month period. Since the variation in the amount of deposit occurred almost entirely in the high-heat section where the deposit consists largely of mineral, he suggested that the variation may be due to variation in the mineral components and that a decrease in mineral content may reduce fouling. However, Burton (1967) found a similar seasonal variation in the amount of deposit was strongly positively correlated with fat content and not the mineral or protein contents of the milk.

Fouling is the cause of one of the most important problems in the dairy industry, the need for constant cleaning of heating equipment that affects the economic efficiency of the plant (due to plant down time, increased fuel and cleaning materials costs, and increased capital cost). It also affects the thermal efficiency of the plant (the deposit on the surface acts as an insulator reducing the rate of heat transfer to the milk) and the quality of milk (the milk may contain detached pieces of the deposits, which may lead to sensory defects) (Nivedita and Hilton, 2007).

2.7.6 Aseptic Processing of Milk

Aseptic processing offers an alternative to conventional canning to meet the demand for convenient and high quality foods. Aseptic processing of foods is a process in which the product and the package are sterilized separately and brought together in a sterile environment. It involves sterilization of a food product, followed by a specified period of time in a holding tube, cooling, and finally packaging in a sterile container. Aseptic processing uses high temperature for a short period of time to yield a high quality product (nutrients, flavor, color, or texture) compared to that achieved with conventional canning. Some of the other advantages associated with aseptic processing include longer shelf life (1-2 years at ambient temperature), flexible package size and shape, less energy consumption, less space requirement, elimination of the need for refrigeration, easy adaptability to

automation, and need for fewer operators. However, some of the disadvantages of aseptic processing include slower filler speeds, higher overall initial cost, need for better quality control of raw ingredients, better trained personnel, better control of process variables and equipment, and stringent validation procedures (David *et al.*, 1996).

2.7.7 Shelf Life of Milk

Paperboard laminate cartons are multilayer containers, usually rectangular with a flat top or tetrahedral shape. For UHT milk packaging applications, aluminum foil is added to the conventional LDPE/paperboard/LDPE structure between the paperboard and the internal LDPE layer (LDPE/paperboard/LDPE/alufoil/LDPE/LDPE) to provide the required barrier properties. The innermost LDPE layer is applied at a lower temperature than the adjacent layer to minimize the tendency for LDPE degradation products formed at high temperatures to diffuse into the milk and alter its taste.

Simon and Hansen (2001b) used (a) standard milk board, (b) standard board including an ethylene vinyl alcohol (EVOH) barrier layer, and (c) standard board including an aluminum foil layer to package 2% UP milk stored at 6.7°C. Quality was assessed over a period of 15 weeks. They found that the flavor of milk packaged in standard board deteriorated at a faster rate than that of milk packaged in barrier and foil boards.

At week 6 of storage, a slightly cardboard flavor was detected in milk packaged in standard board, and a slightly cooked flavor was detected in milk packaged in barrier and foil boards. The cardboard flavor intensified with storage time, but the cooked flavor had dissipated by week 10 of storage.

Rysstad and Kolstad (2006) described the Pure-Lac system developed by Elopak (Spikkestad, Norway) and APV (Silkeborg, Denmark) in the mid-1990s. The Pure-Lac plant is almost identical to the direct-heated steam-infusion UHT plant but is operated at conditions designed to kill heat-resistant psychrotrophic aerobic spores without damaging milk flavor. Holding conditions are 130–145°C for <1 sec with infusion heating and flash-cooling times of <0.2 and <0.3 sec, respectively. Holding conditions and packaging options (clean, ultraclean, or aseptic filling) can be tailored to suit the flavor profile and shelf life required by the processor (Value and Castberg, 1991). A shelf life of >45 days at 10°C can be achieved in plastic /alufoil / paperboard laminate cartons, which provide zero light transmission.

Farrer (1983) compared UHT milk packaged in LDPE-coated paperboard cartons with and without an aluminum foil layer. Results showed that O_2 in the milk packaged in the container with aluminum foil remained almost unchanged at 1 ppm after 44 days, whereas in the milk packaged in the container without aluminum foil, O_2 rose to 8-9 ppm after only a few days. Milk in the carton containing aluminum foil was organoleptically acceptable for 2 months even when stored at 38°C, whereas in the carton without foil, the milk was acceptable only for up to 3 weeks when stored at 15°C.

Rysstad *et al.* (1998) evaluated the sensory and chemical shelf life of UHT milk stored at room temperature and 6°C in 1 L gable-top cartons with three structures: an aluminum foil barrier; a non-foil, paper-based barrier (X-board); and LDPE-coated paperboard. The OTR of the three structures was 0, 2–4, and >200 ml O_2 m^{-2} day^{-1}, respectively, but, unfortunately, the surface area of the car-tons was not given. UHT milk in cartons with an aluminum foil barrier layer stored in the dark had a shelf life of 6 months, whereas milk stored in the X-board and LDPE-coated cartons had a shelf life of 4–5 months. When LDPE-coated and X-board cartons were stored under direct light exposure at 6°C, a light-induced off-flavor was detected after 2 and 8 weeks, respectively. The light-induced off-flavor effect was more pronounced than the effect of autoxidation of unsaturated lipids.

Shelf life is the age by which the quality of fluid milk changes from acceptable to unacceptable. The shelf life of fluid milk is impacted by several different factors. The use-by (UBD) and sell-by dates (SBD) are an estimation of shelf life. These dates may be used by the consumer to determine when milk will spoiled. By law, fluid milk must have a date printed on the container. However, it is the processor who decides how long the code date lasts. The law does not prohibit the sale of fluid milk that has surpassed its SBD or UBD. The total plate count is another benchmark for milk spoilage and has been used for decades. Pasteurized Grade "A" milk is required to have less than 20,000 cfu/ml total count and less than 10 cfu/ml for the coliform count (FDA, 2009). However, freshly pasteurized milk often contains less than 500 cfu/ml. Raw milk from a single producer must contain less than 100,000 cfu/ml total count and less than 300,000 cfu/ml total count if it is comingled (FDA, 2009). Although these regulations help to maintain good quality milk, bacterial growth is not the only cause of milk spoilage. Off –flavors in fluid milk can arise from many different root causes such as chemical reactions and absorption of off-flavors from the environment. Sensory evaluation is another tool for determining milk spoilage. With a combination of sensory methods one could determine when a milk samples has spoiled and define the off-

flavors potentially giving clues as to what the cause of spoilage may have been. These methods range from descriptive analysis panels, which can describe and characterize off-flavors in a product, to consumer acceptability testing, and discrimination testing. Currently, fluid milk processors taste the milk when the UBD or SBD is up to determine if they are meeting their benchmarks. This method does not prevent inferior quality milk from being sold. The sale of reduced quality milk has a negative effect on the consumer's perception of the product and the dairy industry as a whole (Wustenberg, 2012).

2.8 Milk Adulteration

Milk adulteration is an act of intentionally debasing the quality of food offered for sale either by admixture or substitution of inferior substances or by the removal of some valuable ingredients (Food & Drug Administration, 1995). Adulterated food is dangerous for health as it may contain various toxic chemicals, it may be deprived of nutrients required for proper growth and development of human body (Marcus, 1979). Milk used by the people for consumption is adulterated to such an extent that there is very less nutritive value in it and may also be toxic for public health their profit margin by three ways dilution, extraction of valuable components like milk fat which is removed as cream, addition of cheap substances like starch to increase the value of total solids up to a level which is acceptable by the consumers. In Bangladesh, raw milk is distributed by a traditional system which involves middlemen called Gowala. These middlemen used to adulterate milk to maximize their profit (Lateef *et al.*, 2009).

Normally, the adulteration in food is done either for financial gain or lack of proper hygienic conditions of processing, storing, transportation and marketing. This ultimately leads to the stage that the consumer is either cheated or often becomes victim of diseases. Such types of adulteration are quite common in developing countries. It is equally important for the consumer to know the common adulterants and their effects on health (Faraz *et al.*, 2013).

Milk adulteration, poor hygiene, malpractices, lack of preservation technology, cooling facilities and sanitation conditions are the main causes of losses in quantity and adulteration of milk is the most pressing public health issue. Adulteration of milk is done to increase its volume and then starch and other reconstituted milk powders are added to increase its viscosity. To increase the shelf life of milk dirty ice and some chemicals like hydrogen peroxide, carbonates, bicarbonates, antibiotics, caustic soda and even the most lethal

chemical formalin is also being used. Urea adulterated milk is very harmful to the girls as it hastens up the process of puberty (Tariq, 2001).

The adulterants/preservatives assume the proportion of health hazards for end consumers, particularly infants (Tipu *et. al.*, 2007). Suppliers of milk appear to have found three ways to increase their margin from the sale of milk: (i) dilution (ii) extraction of valuable components, i.e. milk fat removed as cream, and (iii) a combination of (i) and (ii) with the addition of cheap (and sometimes potentially harmful) bulking additives, such as low quality flour, to bring the total solids to a level which is acceptable to consumers. Some of the chemicals, adulterants and malpractices results in public health concern and malnutrition (Faraz *et al.*, 2013).

The chemicals which are being used as adulterants in milk have the following effects on the health of consumers; Formalin causes vomiting, diarrhea and abdominal pain. Larger doses may cause decreased body temp, shallow respiration, weak irregular pulse and unconscious. It also affects the optic nerve and cause blindness. It is one of the potent carcinogens (Gwin *et al.*, 2009). Hydrogen peroxide damages the stomach cells, which can lead to gastritis and inflammation of the intestine and bloody diarrhea (Murthy *et al.*, 1981). High amounts of starch may cause diarrhea due to the effects of undigested starch in colon. Its accumulation in the body may prove very fatal for the diabetic patients. High amounts of carbonates or bicarbonates in the body potentially disrupt hormones signals that regulate development and reproduction (Rideout *et al.*, 2008).

Milk is produced throughout the year. However, milk production is greatly reduced during summer months due to heat stress and scarcity of fodder etc. Milk is transported from point of production to cities mainly through middlemen called "dodhies". Such milk is watered or skimmed to increase profit. To maintain its composition, starch, flour, urea, cane sugar, vegetable oil, etc., are added as adulterants. Milk is a perishable commodity so during summer months, it is likely to be spoiled during transportation. The middlemen therefore add chemical preservatives such as penicillin, strepto-penicillin, formaldehyde, hydrogen peroxide, sodium bi-carbonate, etc. The major problem in the fluid milk supply system in Pakistan from the consumer point of view is not only adulteration but also dirty adulteration. Public consume fluid milk which has been adulterated and diluted to an extent that there is very little nutritive value left in it, resulting, to a great extent, to general public health concerns and malnutrition (Faraz *et al.*, 2013).

CHAPTER III
MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The study was conducted in the laboratory of the Department of Food processing and Preservation and the Department of Dairy and poultry science, Hajee Mohammad Danesh Science and Technology University, Dinajpur. In this chapter determination of physico-chemical quality parameters and adulteration of UHT milk have been discussed.

3.1 Collection of Samples

Four common commercial UHT milks those are marketed in the form of tetra pack were collected from local bakery and confectionery shops. The samples were then transported to the laboratory and stored at room temperature for further analysis. It is noted that every sample was tested with six replications for each quality analysis.

3.2 Physico-Chemical Quality Test of the Samples

The physico-chemical test of the samples was analyzed for fat, protein, water content, solids-not fats (SNF), titratable acidity and pH by Hassan *et al.* (2009) and Awan *et al.* (2013). However, Ibrahim *et al* (2012), Tola *et al.* (2007) and Abdel-Hameid (2002) were analyzed the specific gravity, total solids, solids non fats and moisture for UHT milk. From the above references the following physico-chemical quality parameters was determined.

3.2.1 Protein Content

The protein was estimated by formal titration method by Davide (1997) following Hassan *et al.* (2009) and Saha and Ara (2012).

Principle

When formaldehyde is added to milk, the free amino groups of the protein react with the carbonyl groups of formaldehyde causing the milk to become acidic. The acidity developed is related to the amount of protein present, which may be measured by titrating with sodium hydroxide (NaOH) using phenolphthalein as an indicator.

Protein can't be determined directly with base due to weak carboxylic group in it. By adding formalin (formaldehyde) that react with NH_2 group and form methelen amino ($N=CH_2$) and carboxylic group COOH can be easily titrated.

$$HOOC\text{-}CHR\text{-}NH_2 + HCHO \rightarrow HOOC\text{-}CHR\text{-}N=CH_2 + H_2O$$

Procedure

At first 10 ml of milk was taken in a conical flask. Moreover, 1ml of phenophthalein and 0.4 ml of potassium oxalate (2%) was added to milk. Then it was allowed to stay for 2 minutes. Meanwhile, the milk was titrated by 0.1 N NaOH while the pink color appeared titration was stopped. The ml of NaOH used for titration was recorded. Again 2ml of 40% formaldehyde added into the flask. After then the solution was titrated against 0.1 N NaOH. Since the pink color appeared the titration was carried out. Hence, the pink color was appeared and the used volume of NaOH was estimated. Therefore, the amount of NaOH needed for 10 ml of milk to neutralize the acid formed after adding formaldehyde was calculated.

Calculation

% protein = ml of 0.1N NaOH needed for 10 ml of milk to neutralized acid formed formaldehyde× 1.7

Where,

1.7 is the factor for converting the observed formaldehyde value into percentage of protein by weight.

3.2.2 Fat Content

Fat content in milk was determined according to Gerber method as described by FAO (1997) following Kleyn *et al.* (2001) and, Hossain and Dev (2013).

Procedure

10 ml of sulphuric acid (specific gravity: 1.82 g/cc) was taken in the butyrometer. Then, 10.75 ml of milk sample was added slowly in the butyrometer containing sulphuric acid to prevent charring and violent reaction with acid. Furthermore, 1 ml of isoamyl alcohol was added to butyrometer. Then, a lock stopper was inserted to butyrometer securely using hand-held key. After locking the butyrometer, the mixture was shaken until all traces of curd

disappear. Therefore, the mixture was then centrifuged for 5 min at 1100 rpm. Meanwhile, the butyrometer was placed in water bath at 65°C for 3 to 4 min. Finally, the fat content was measured according to butyrometer scale.

3.2.3 Titratable Acidity Content

The titratable acidity was determined according to O'Mahoney (1988), Aggarwala and Sharma (1961), and Saha and Ara (2012).

Procedure

At first 10 ml of milk was taken in a conical flask. Moreover, 3-4 drops of phenophthalein was added to milk. Then, the milk was titrated by 0.1 N NaOH. As while, the pink color appeared the titration was stopped. Finally, the ml of NaOH used for titration was recorded. The acidity was calculated by the following equation-

$$\text{Titrable acidity} = \frac{\text{Volume of 0.1N NaOH used}(\text{ml})}{\text{Weight of sample}(\text{ml})}\%$$

3.2.4 pH Content

The pH value of milk was measured by using a digital pH meter (HI 8314, Hanna Instruments, Italy) as described by AOAC (2005). At first, the electrode assembled of the pH meter was dipped into the standard buffer solution of pH 7.0 before using the instrument. After the standardization, the electrode assembled pH meter was dipped into the milk sample. Then, the pH was readout in the digital meter of the instrument. After that, the electrode was washed twice with distilled water and dried with tissue paper before measuring the next experiment for pH value with the instrument.

3.2.5 Corrected Lactometer Reading (CLR)

The lactometer test is designed to detect density of milk. It was measured according to Aggarwal and Sharma (1961), Tessema and Tibbo (2009), and Ibrahim *et al.* (2012).

Procedure

The milk samples were mixed gently and poured into a measuring cylinder of 500 ml. The lactometer was then dipped slowly into the cylinder. After sinking, the lactometer reading (LR) of the milk was recorded which was just above the surface of the meter. Meanwhile,

the temperature of the milk was observed through a thermometer. If the temperature of the milk is different from the calibrated temperature (Calibration temperature=20^0C) of the lactometer then it was needed to calculate the temperature correction.

3.2.6 Specific Gravity

Specific gravity test was performed by using Quenvnne lactometer and floating dairy thermometer (Aggarwal and Sharma, 1961). The specific gravity of milk is calculated by the following formula:

$$\text{Specific gravity of milk} = 1 + \frac{\text{CLR}}{1000}$$

Where,

CLR= Corrected Lactometer Reading.

3.2.7 Solids Not Fat (SNF) %

Solids not fat (SNF) % was determined by lactometeric method (Ramsey and Swartzel, 1984; Hassan *et al.*, 2009 and Ibrahim *et al.*, 2012). As calculated by the following formula-

$$\text{SNF} = \frac{\text{Fat\%}}{5} + \frac{\text{CLR}}{4} + 0.14$$

Where,

CLR= Corrected Lactometer Reading.

3.2.8 Total Solids (TS)

The milk total solids (TS) percentages of examined UHT milk samples were calculated according to Ling (1963), Ibrahim *et al.* (2012) and Imran *et al.* (2008). The total solids of the samples was calculated by the following formula-

$$\text{TS} = (\text{Fat\%} \times 1.2) + \frac{\text{CLR}}{4} + 0.14$$

Where,

CLR= Corrected Lactometer Reading.

3.2.9 Water Content

Water content was calculated by subtracting the total solids percentages from the weight of the original samples (Abdel-Hameid, 2002; Ling, 1963 and Ibrahim *et al.*, 2012).

3.2.10 Clot on Boiling Test (COB)

Approximately 2ml of milk sample was taken in a test tube and treated over sprit lamp. Then it was allowed to boil for 1 to 2 minutes. After that it was observed that whether the milk sample was clotted /coagulated or not in the test tube (Tessema and Tibbo, 2009).

3.2.11 Alcohol Test

Ethanol solution (68 %) was prepared from 68 ml of 96% (absolute) alcohol with adding 28 ml of distilled water. Then the test was conducted by mixing equal amounts of milk and 68% of ethanol solution in a test tube (Tessema and Tibbo, 2009). Therefore, if the milk was not clotted, coagulated or precipitated the milk having with good quality.

3.3 Detection of Adulterants in the Milk Sample

Various milk adulterants such as added water, starch, formalin, hydrogen peroxide, detergents, and cane sugar was observed by Faraz *et al.* (2013), Ibrahim et al (2012), Hossain *et al.*(2011), Tipu *et al.* (2007), Lateef *et al.* (2009), and Khan *et al.* (1999). From the above references the following adulterants that may present in UHT milk sample was detected.

3.3.1 Added Water Content

The added water content was calculated according to the following equation (Ibrahim *et al.*, 2012) given in below-

$$\text{Percentage of water added} = \frac{\text{Milk solids not fat \% legal - Milk solids not fat \% of sample}}{\text{Milk solids not fat \% legal}} \times 100$$

3.3.2 Hydrogen Peroxide

Hydrogen peroxide is intentionally added in milk which prolongs the shelf life of milk. The hydrogen peroxide of UHT milk sample was determined according to Faraz *et al.* (2013) and Tipu *et al.* (2007).

Procedure

At first 5ml milk was taken in a caped test tube. Then 0.5 ml (5%) of KI solution, 0.5 ml of 2% starch solution and 5 ml of concentrated HCl acid were added and mixed into the test tube containing milk sample. After that the test tube was closed with cap and putted in invert position for 2 to 3 min. Therefore, the change of color of the mixture was observed. If the color of the mixture was changed to blue ash, the sample indicated the presence of H_2O_2.

3.3.3 Rosolic Acid Test (Soda)

Five (5) ml of milk was taken in a test tube. Then 5 ml alcohol was added followed by 2-3 drops of rosolic acid. Therefore, the change of color of the mixture was observed. If the color of milk changed to pinkish red then it indicated that the milk was adulterated with sodium carbonate/sodium bicarbonate (dairyforall.com/milk-adulteration/2013).

3.3.4 Formaldehyde

At first 10 ml of milk was taken in a test tube. Then 5 ml concentrated H_2SO_4 was added through the sides of the test tube without shaking it. Hence, the change of color was observed. If a violet or blue ring appears at the intersection of the two layers, it shows the presence of formalin (dairyforall.com/milk-adulteration/2013).

3.3.5 Starch

Three (3) ml milk was taken in a test tube and boiled it over sprit lamp. Then, the milk was cooled to room temperature. After that, 2 to 3 drops of 1% iodine solution was added into the milk. Therefore, the change of color was observed. If the color of the milk changed to blue then it indicated that the milk was adulterated with starch (dairyforall.com/milk-adulteration/2013).

3.3.6 Cane Sugar

At first 10 ml of milk was taken in a test tube. Then 5 ml of hydrochloric acid was added along with 0.1 g of resorcinol in the test tube containing milk sample. Then the test tube was shaken and placed the test tube in a boiling water bath for 5 min. If the color of the test tube mixture was changed to red color which indicated that the presence of added sugar/sucrose in milk (dairyforall.com/milk-adulteration/2013).

3.3.7 Carbonates

At first 5 ml of milk was taken in a test tube. Then 5 ml of alcohol was added with few drops of an alcoholic solution of Rosalic acid (1% w/v) and mixed properly. If carbonate is present a rose red color appears whereas pure milk shows only a brownish coloration (Faraz *et al.,* 2013).

3.3.8 Skim Milk Powder

At first 2 to 3 ml of UHT milk sample was taken in a test tube. Then nitric acid was added drop by drop into the test tube. After adding the acid, if orange color formed it indicated that the milk is adulterated with skim milk powder. However, if yellow color observes then the milk sample is not adulterated with skim milk powder (Awan *et al.*, 2013).

3.3.9 Sodium Chloride

Five (5) ml of silver nitrate salt (0.8%) was taken in a test tube. Then, 2 to 3 drops of 1% potassium dichromate was added with 1 ml of UHT milk sample. After that the mixture was mixed properly. Therefore, if the mixture turns into yellow color then the milk is adulterated with salt. On the contrary, if chocolate color forms then the milk is free from salt (Awan *et al.*, 2013).

3.3.10 Pulverized Soap

Ten (10) ml of milk was taken in a test tube and diluted it with equal quantity of hot water. Then, 1-2 drops of phenolphthalein indicator was added. After adding phenolphthalein indicator, if pink color is formed it indicates that the milk is adulterated with soap (Awan *et al.*, 2013).

3.4 Statistical Analysis

The data for the quality parameters of the present study were statistically analyzed. All the analyses were conducted single factor complete randomized design (CRD). The analysis of variance (ANOVA) for different quality parameters were performed by MSTAT-C statistical computer package while means were compared by the Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

CHAPTER IV
RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Physico-Chemical Parameters of UHT Milk

4.1.1 Protein Content

The percentages of protein content of different UHT milk with standard value were shown in Figure 4.1. The values of different UHT milk obtained from RD, PRAN, Farm Fresh and Aarong were (3.372±0.13) %, (3.202±0.13) %, (3.417±0.13) % and (3.258±0.13) % respectively. The six independent results were shown in Appendix 2. Analysis of variance (ANOVA) (Appendix 3) was carried out for different UHT milk with the standard value (BSTI, 2002). Protein content of four UHT milk samples like RD, PRAN, Farm Fresh and Aarong were found statistically significant against control i.e. standard value of protein (3.30%). This indicates that different brand UHT were not equally acceptable on the basis of protein content (Appendix 4). The maximum protein was recorded 3.417% from Farm Fresh UHT milk and the minimum (3.202%) from PRAN. From the Figure 4.1 protein content from RD was moderate (3.372%). The result showed that incase of protein content Farm Fresh UHT milk was produced the maximum value but RD UHT milk was found standard. It was also observed that the protein content of PRAN (3.202%) and Aarong (3.258%) does not fulfill the BSTI (2002) standard. The standard value has been shown in Appendix 1. Hossain *et al.* (2011) was reported that the higher protein content in 3.43%, 3.52% and 3.68% for different UHT milk samples. Hassan *et al.* (2009) was observed the maximum value of protein 3.70% and minimum 3.30% for different UHT milk. The proteins of milk are the constituents mostly affected by heating and subsequent storage of milk. The principal changes in UHT milk during storage may be due to enzymes. Many proteins in milk are very heat labile e.g. whey proteins, vitamin binding proteins, antimicrobial proteins etc. (Biryunkova *et al.*, 1975). These proteins coagulate after heating hence the texture of milk is deteriorated during storage (Fox and McSweeny, 1998). Casein polymerization is greater at high storage temperature, but occurs significantly even under refrigeration: 50% of the protein may be in the polymer form after 6 months at 37°C and 21% after 6 months at 4°C (Andrews *et al.*, 1977).

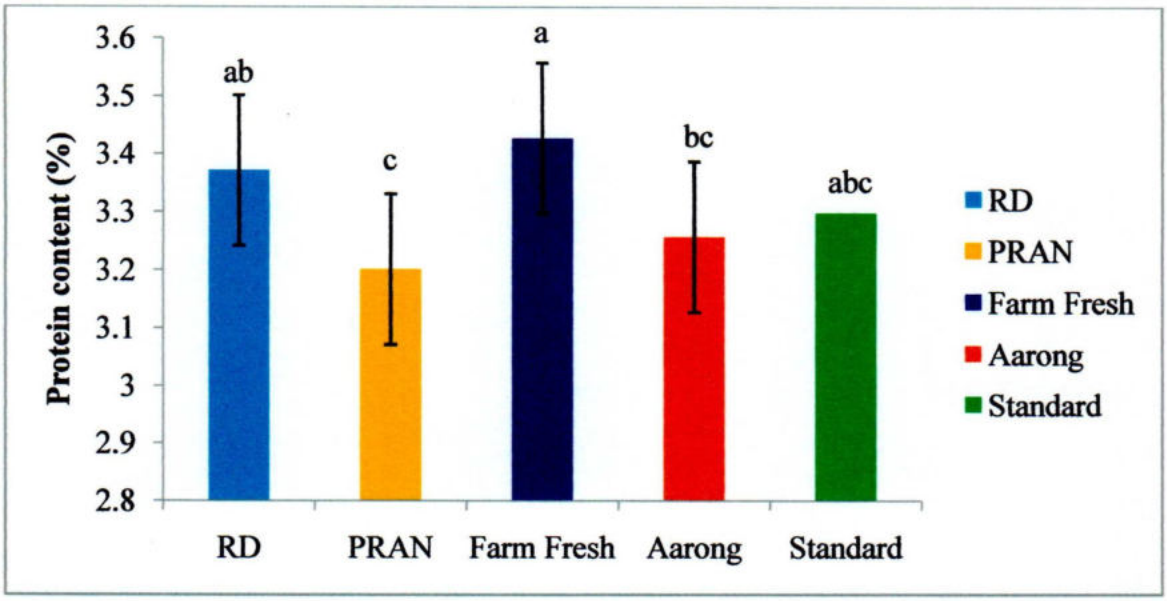

Figure 4.1: Protein content of different UHT milk.

The protein content of the raw milks varied from 3.07% to 3.57%. Lingathurai *et al.* (2009) reported slightly higher (3.77%) protein content. Two UHT milk samples were RD and Farm Fresh satisfied this requirement each containing a minimum of 3.37% protein and others two (PRAN and Aarong) samples dose not fulfill the above requirement. But each UHT milk processing company labeled into the package protein content is 3.4%. From the DMRT test (Appendix 4); only one UHT milk processing company (Farm Fresh) maintained that standard.

4.1.2 Fat Content

Figure 4.2 shows the fat percentages of UHT milk obtained from RD, PRAN, Farm Fresh and Aarong value were (3.133±0.08)%, (3.40±0.17)%, (3.50±0.09)% and (3.50±0.09)%, respectively. The six independent results are shown in Appendix 5. Analysis of variance (ANOVA) (Appendix 6) was carried out for different brands of UHT milk with standard fat content. Statistical analysis revealed that there was significant ($p<0.05$) difference within the fat content of different brand's UHT milk. This indicates that different brand's UHT were not equally acceptable on the basis of fat percentage. It was also observed that the average value of fat obtained from Farm Fresh and Aarong was same value (3.50±0.09%), significantly higher than the fat content of PRAN (3.40%) and RD (3.133%). It was also observed that the average value of fat obtained from RD (3.133±0.08) % shows statistically (DMRT) lower fat content of other three samples (Appendix 7). The BSTI (2002) requirement for fat content of pasteurized milk is a minimum of 3.5% which standard is

followed by the dairy industries for UHT milk. But each UHT milk processing company labeled into the package fat content is 3.50%. From the DMRT test (Appendix 7); two UHT milk processing company (Farm Fresh and Aarong) maintained that standard (3.50%) among the tested UHT milk sample.

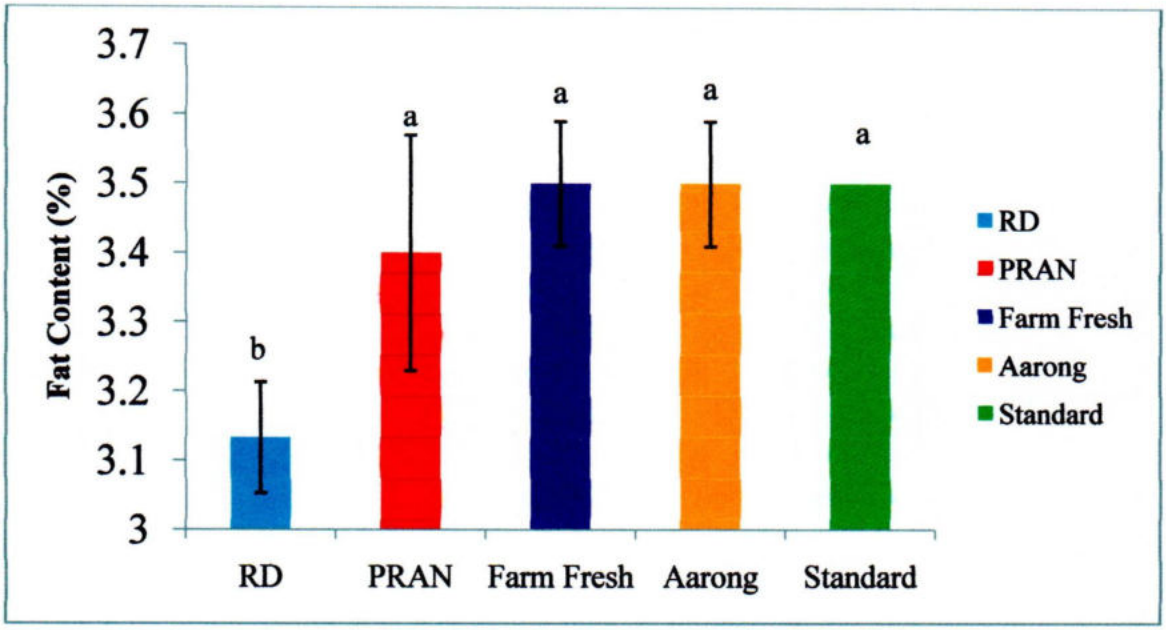

Figure 4.2: Fat content of different UHT.

Two samples like Farm Fresh and Aarong (3.50%) were fulfilled the mentioned standard (3.50%) and others two UHT milk samples (RD and PRAN) fat content were below the standards. The standard value has been shown in Appendix 1. Hossain *et al.* (2011) found that the fat content of UHT milk: one of the UHT-processed milks was even less (3.09%) and another high (3.62%); also found maximum (3.75%) and minimum (3.12%) for the raw milk. Hassan *et al.* (2009) was reported that the higher value of fat content of different UHT milk between 3.50% and 3.80%. Milk composition varies considerably among breeds of dairy cattle (Zinash *et al.*, 1988; Chamberlain, 1990) According to O'Connor, (1994); Jersey and Guernsey breeds give milk with about 5.0% fat while the milk of shorthorns and Friesians contains about 3.5% fat.

4.1.3 pH Content

The pH content of different UHT milk obtained from RD, PRAN, Farm Fresh and Aarong were 6.30±0.09, 6.10±0.09, 6.133±0.10 and 6.217±0.08, respectively. The six independent results are shown in Appendix 8. Analysis of variance (ANOVA) (Appendix 9) was carried out for pH value of those samples and results revealed that there were significant ($p<0.05$)

differences in pH value among the samples. This indicates that the pH content of different UHT milk was not equally acceptable. As shown in Appendix 10 (DMRT) the RD milk was secured the highest value for pH (6.30) than other samples. The pH of normal, fresh, sweet milk usually varies from 6.4 to 6.6 (Sukumar, 2005). None of the samples fulfilled that standard (6.50). The standard value has been shown in Appendix 1. Figure 4.3 shows the pH value of four UHT milk sample viz RD, PRAN, Farm Fresh and Aarong against control i.e. standard value of pH.

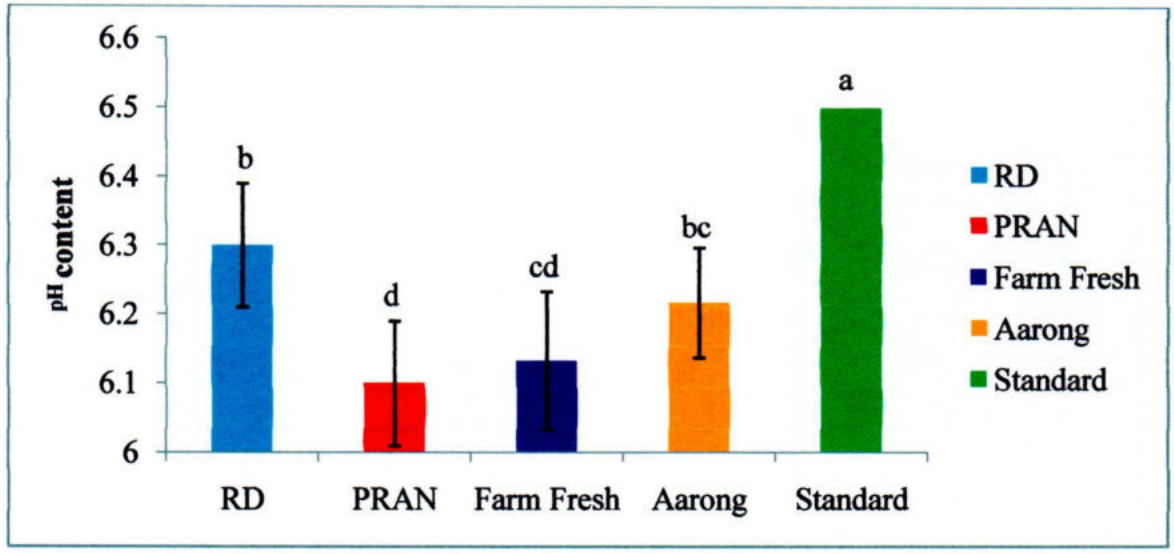

Figure 4.3: pH content of different UHT milk.

From the Figure 4.3 we can conclude that PRAN has the lowest pH (6.10) value. It also concludes that none of the UHT milk sample does not fulfill the standard pH (6.5). pH is the parameter that determines the sample acidity and alkalinity. Hassan *et al.* (2009) found that the pH was decreased during storage of UHT milk; the decreased in pH was 6.81 to 6.20 (first week to 12th week), respectively. Awan *et al.* (2013) found the pH content was 6.00 of different UHT milk samples. Rehman and Salaria (2005) found that the pH content range was 6.38±0.60 to 6.77±0.88 for UHT processed cow milk. Processing operations influence acid base equilibrium in milk. UHT treatment results in a pH decrease, due to conversion of lactose into different organic acids (Fox and McSweeny, 1998). Vankatachalm and McMahon (1991) verified a drop in pH and they associated it with browning reactions. Andrews *et al.* (1977), confirmed similar effects and concluded that the level and extent of pH decrease was related to age-gelation. When milk is heated at a temperature above 100°C and subsequently stored, lactose is degraded to acids. Formic acid is the principal acid

produced due to storage which is titratable acidity of milk rises. Increase in free fatty acids is also responsible for increasing the total titratable acidity of milk (Swartzel, 1983).

4.1.4 Water Content

The water content obtained from RD, PRAN, Farm Fresh and Aarong were (89.13±0.12)%, (88.69±0.29)%, (88.64±0.12)% and (88.67±0.10)%, respectively. The six independent results are shown in Appendix 11. Analysis of variance (ANOVA) was carried out for different brands of UHT milk against standard value of water content (Appendix 12). Figure 4.4 reveal the water content of different UHT milk against standard value.

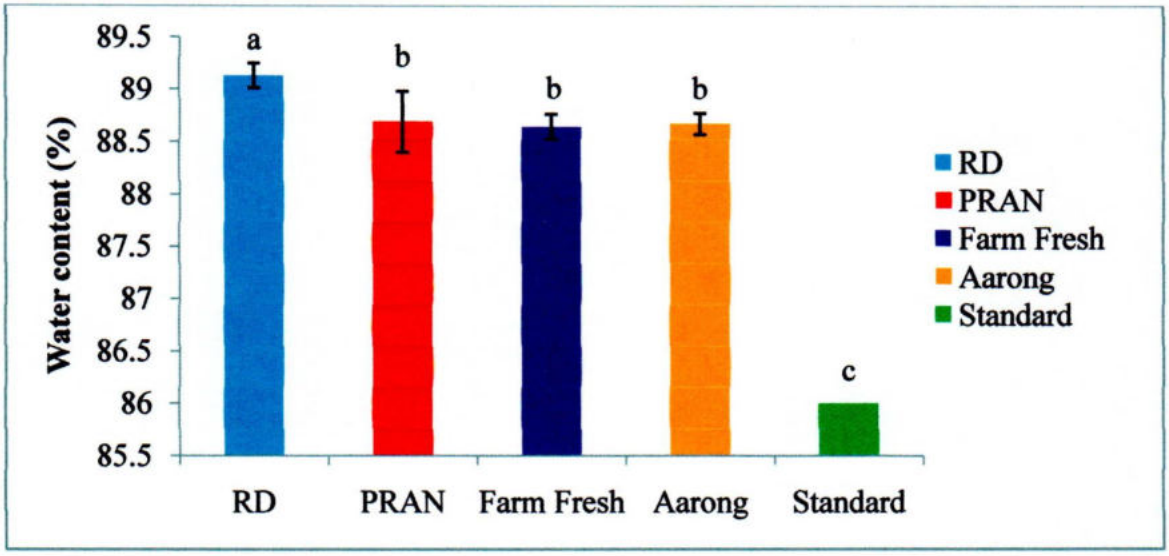

Figure 4.4: Water content of different UHT milk.

Statistical analysis revealed that there was significant ($p<0.05$) difference within the water content of different UHT milk. The DMRT test (Appendix 13) indicated that different UHT were not equal in water / moisture content. Results showed that maximum water content was recorded in RD UHT milk (89.13%) whereas PRAN UHT milk (88.69%) and Aarong UHT milk (88.67%), while minimum value was recorded in Farm Fresh UHT milk (88.64%). The water content of different UHT milk was above the standard value (86.0%) as shown in Appendix 1. Hossain *et al.* (2011), found the water content in UHT milk which was 88.0% to 89.0%, similar to that reported by Hossain *et al.* (2010). The water content of raw milk within the standard value (86.8%) was observed by Imran *et al.* (2008). The difference in water content might be due to the difference in feeding and breed (Nickerson, 1960). The usual range of water content in milk is 84.0 to 89.0% (Eckles *et al.*, 1951) and above the range, milk was adulterated with water.

4.1.5 Solids-Not-Fat (SNF) Content

Solids-not-fat (SNF) percentage of UHT milk obtained from RD, PRAN, Farm Fresh and Aarong were (7.742±0.06) %, (7.793±0.03) %, (7.865±0.09) % and (7.832±0.07) %, respectively. The six independent results are shown in Appendix 14. Analysis of variance (ANOVA) was carried out for different brands of UHT milk (Appendix 15). Statistical analysis revealed that there was significant ($p<0.05$) difference within the SNF content of different UHT milk (Appendix 16). This indicates that different UHT were not equally acceptable on the basis of SNF percentage. FDA standard for SNF content of whole milk is a minimum of 8.25% (Graf, 1976). None of the UHT processed milk maintained this standard. BSTI (2002) standard for SNF content of pasteurized milk is a minimum of 8.00%, which standard is followed by all of the dairy industries for UHT milk in Bangladesh. The standard value has been shown in Appendix 1. From the present study it was observed that SNF content of all of the UHT milk samples were of >7.00% and was near to 8.00%. Hossain *et al.* (2010), found the higher content of SNF which was 8.56% for UHT milk. Hossain and Dev (2013) found the SNF content of raw milk was 7.81%. The mean value of SNF of different UHT milk is shown in Figure 4.5.

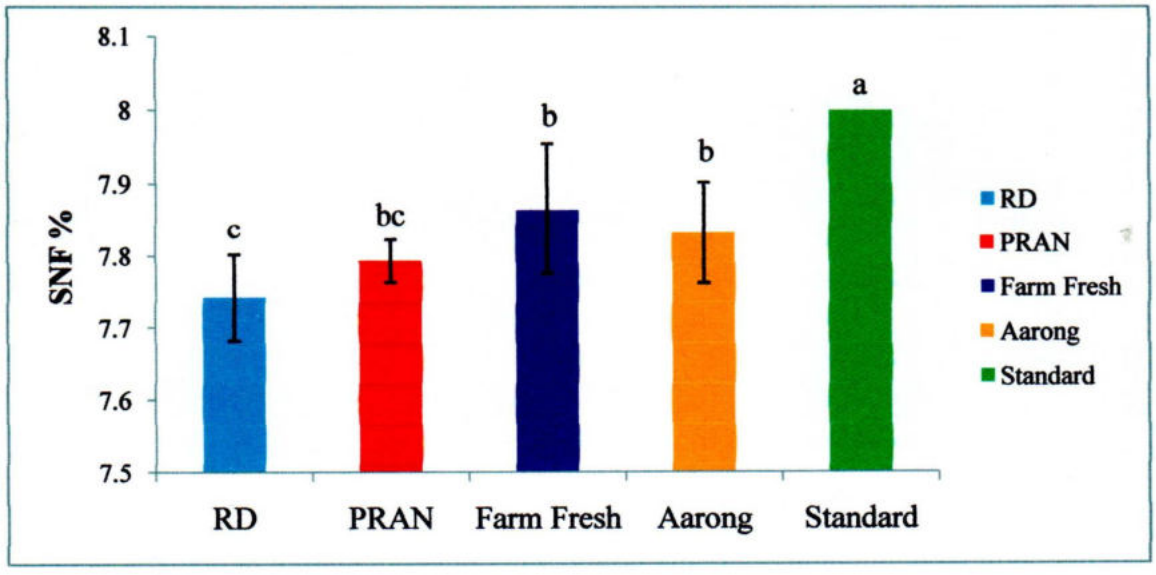

Figure 4.5: SNF content of different UHT milk.

4.1.6 Titratable Acidity

Titratable acidity of UHT milk obtained from RD, PRAN, Farm Fresh and Aarong were 0.1717±0.01, 0.1917±0.01, 0.1833±0.01 and 0.1833±0.01, respectively (Table 4.1). The six

independent results are shown in Appendix 17. According to BSTI (2002), for the UHT milk the maximum titratable acidity is 0.15% (Appendix 1). For a longer shelf life, the acidity of milk should be less than 0.15 % as indicated in BDS (1985). Results of the present study were above according to the BSTI (2002) standard. According to Richardson, (1985); O'Connor, (1994) the percentage of acid present in dairy product at any time is a rough indication of the age of milk and the manner in which it has been handled.

Titratable acidity is a measure of freshness and bacterial activity in milk. Popescu and Angel (2009) reported that high quality milk has to have less than 0.14 percent acidity. The acidity of the raw milk samples varied largely from one sample to another. The highest value was 0.1917 % (PRAN) indicating high bacterial activity prior to heat treatment and the lowest was 0.1717% (RD) indicating it's relatively better quality with regards to freshness. Hossain *et al.* (2010) found that there were no bacteria present in the UHT milk, but both those sample showed high degree of titratable acidity (0.189% and 0.175% respectively) suggesting that the high acidity might have developed prior to the heat treatment. Hassan *et al.* (2009) found that the titratable acidity was increased during storage of UHT milk; the increased in acidity was 0.11% to 0.18% (first week to 12th week), respectively. Within a shorter period of milking the acidity increases perceptibly due to lower bacterial activity. The degree of bacterial contamination and the temperature at which the milk is kept are the chief factors influencing acid formation. Therefore, the amount of acid depends on the cleanliness of production and the temperature at which milk is kept. For this reason, determination of acid in milk is an important factor in judging milk quality. Acidity affects taste as well. When it reaches about 0.3%, the sour taste of milk becomes sensible. At 0.4% acidity, milk is clearly sour, at 0.6% it precipitates at normal temperature and acidity over 0.9% moulds might grow (Tzouwara-Karayanni, 2000).

Table 4.1: Summary of the results of physico-chemical parameters of UHT milk samples available in local market

Parameters	RD	PRAN	Farm Fresh	Aarong	Standard
Sp. Gr.	1.027±0.0002	1.0278±0.0002	1.0281±0.0003	1.0279±0.0002	1.028
Titatable acidity (%)	0.1717±0.01^{a}	0.1917±0.01^{a}	0.1833±0.01^{a}	0.1833±0.01^{a}	0.150±0.0^{a}
TS	10.88±0.12^{c}	11.31±0.29^{b}	11.36±0.12^{b}	11.33±0.10^{b}	12.00±0.0^{a}
CLR	27.90±0.21^{a}	27.87±0.24^{a}	28.10±0.37^{a}	27.97±0.29^{a}	28.0±0.0^{a}

4.1.7 Corrected Lactometer Reading (CLR)

Corrected lactometer reading of UHT milk obtained from RD, PRAN, Farm Fresh and Aarong were 27.90±0.21, 27.87±0.24, 28.10±0.37 and 27.97±0.29 respectively (Table 4.1). The six independent results are shown in Appendix 18. A two way analysis of variance (ANOVA) (Appendix 19) was carried out for different UHT milk brands. Statistical analysis revealed that there was non-significant difference within the CLR of different UHT milk. The standard CLR for milk is 28.0 to 30.0 (Sukumar, 2005). The results showed that only one UHT milk sample Farm Fresh (28.1) fulfilled that standard and others milk samples almost near to the value (28.0). The standard value has been shown in Appendix 1. Lower in CLR value indicates that the milk was adulterated with water.

4.1.8 Specific Gravity

Specific gravity of UHT milk obtained from RD, PRAN, Farm Fresh and Aarong were 1.0279±0.0002, 1.0278±0.0002, 1.0281±0.0003 and 1.0279±0.0002, respectively (Table 4.1). The six independent results are shown in Appendix 20. It was found that there were non-significant differences within the specific gravity of different types of UHT milk. Therefore on the basis of specific gravity all samples maintain the same quality. The standard value for specific gravity is 1.028 minimum (BSTI, 2002). The standard value of specific gravity range of 1.028 to 1.033 (FAO, 1990). Also from the present study it was observed that the specific gravity of UHT milk were almost within the normal range.

4.1.9 Total Solids (TS) Content

Total solids (TS) percentage of UHT milk obtained from RD, PRAN, Farm Fresh and Aarong were 10.88±0.12, 11.31±0.29, 11.36±0.12 and 11.33±0.10, respectively (Table 4.1). The six independent results are shown in Appendix 21. Analysis of variance (ANOVA) was carried out for different brands of UHT milk (Appendix 22). Statistical analysis revealed that there was significant ($p<0.05$) difference within the total solids content of different UHT milk. The DMRT test (Appendix 23) indicates that different brand's UHT were not equally acceptable on the basis of total solids content. It was also observed that the average value of TS obtained from Farm Fresh (11.36±0.12%) was significantly higher than Aarong (11.33±0.10%) and PRAN (11.31±0.29%). The RD milk sample showed significantly lower TS (10.88±0.12%) than other three samples. In this study it was concluded that the different UHT brands did not maintain the standard of TS content. The standard value has been shown

in Appendix 1. Hossain *et al.* (2011) was reported that the higher TS content 12% and lower 11% in different UHT milk samples. The TS content of different Pasteurized milk samples obtained from Milk vita, Tatka, Farm Fresh, Aarong and RD were 11.46%, 11.58%, 11.41%, 11.53% and 11.35%, respectively (Saha and Ara, 2012). Addition of water dilutes milk and reduces its TS content. Reduced TS was observed in five raw and one pasteurized milk; none of these samples had TS over 9.5% though milk TS usually ranges from 10.5 to 14.5% (O'Mahony 1988). Hossain *et al.* (2011) found that the UHT-milks were comparatively rich in TS content and the value was at least 11.0% TS. The total solids in the milk ranged from 10.0% to 17.0%, which include fat and non-fat materials. The amount of fat materials is 3.0% to 4.0% and the amount of non-fat material is in the range of 7% to 10% (Webb *et al.*, 1974; Hassan, 2005).

4.1.10 Clot on Boiling Test

In this study, the different UHT milk showed the negative result which mean that the milk did not clot on heat treatment (i.e. pasteurization or UHT). Data were presented in Table-4.2.

Table 4.2 Test results of COB and Alcohol for UHT Milk

Milk Sample	COB Test	Alcohol Test
RD	-	-
PRAN	-	-
Farm Fresh	-	-
Aarong	-	-
"-" =Negative		

4.1.11 Alcohol Test

All the UHT milk showed the negative result on alcohol test which means the milk was good quality i.e. not sour. Data have been presented in Table 4.2. The alcohol test is useful as an indication of the mineral balance of milk and not as much as an index of developed acidity. The test aids in detecting abnormal milk, such as colostrums, milk from animals in late lactation, milk from animals suffering from mastitis and which the mineral balance has been disturbed.

4.2 Adulteration in Milk

The collected UHT milk samples were analyzed for various adulterants i.e. added water, hydrogen peroxide soda, formaldehyde, starch, cane sugar, carbonate, skim milk powder, sodium chloride and pulverized soap.

In milk industry, a preservative means a substance which when be added to milk, will retard sourness or decomposition. The object of adding these preservatives being: to prolong the period of sweetness of milk, to inhibit and to destroy bacteria, and to neutralize acids formed by bacteria and to delay curding. A common form of milk adulteration has been occurred by addition of inhibitory substances and preservatives (Ibrahim *et al.*, 2012). The summary of the tested results of adulteration are shown in Table 4.3.

Table 4.3: Summery of the results of adulteration test of UHT milk sample

Adulterants	**RD**	**PRAN**	**Farm Fresh**	**Aarong**
Added water	+	+	+	+
Hydrogen per oxide	-	-	-	-
Rosolic acid	-	-	-	-
Formaldehyde	-	-	-	-
Starch	-	-	-	-
Cane sugar	-	-	-	-
Carbonates	-	-	-	-
Skim milk powder	-	-	-	-
Sodium chloride	-	-	-	-
"+" = Positive and "-" =Negative				

4.2.1 Added Water Content

A finding could be attributed to adulteration by addition of water and so, it may lead to decrease in legal requirements of fat content and SNF percentage, as well as, normal values of Sp. Gr. and TS (Table 4.1). Added water percentage of UHT milk obtained from RD, PRAN, Farm Fresh and Aarong were 3.229±0.73%, 2.583±0.43%, 1.688±1.14% and 2.104±0.84%, respectively. Figure 4.6 pointed out that UHT milk samples collected from market was adulterated with water since moisture content and SNF content are run near to parallel of BSTI, (2002) regulated standards. The six independent

results are shown in Appendix 24. A two way analysis of variance (ANOVA) was carried out for different brands of UHT milk (Appendix 25). DMRT test (Appendix 26) revealed that there was significant ($p<0.05$) difference within the added water percentage of different UHT milk brand. This indicates that different brand UHT were not equally acceptable on the basis of added water percentage. Faraz *et al.* (2013) was found that 97% and 93% of the raw milk samples collected from canteens of educational institutes and public places showed water addition in them out of 60 samples which was collected for at least 6 weeks.

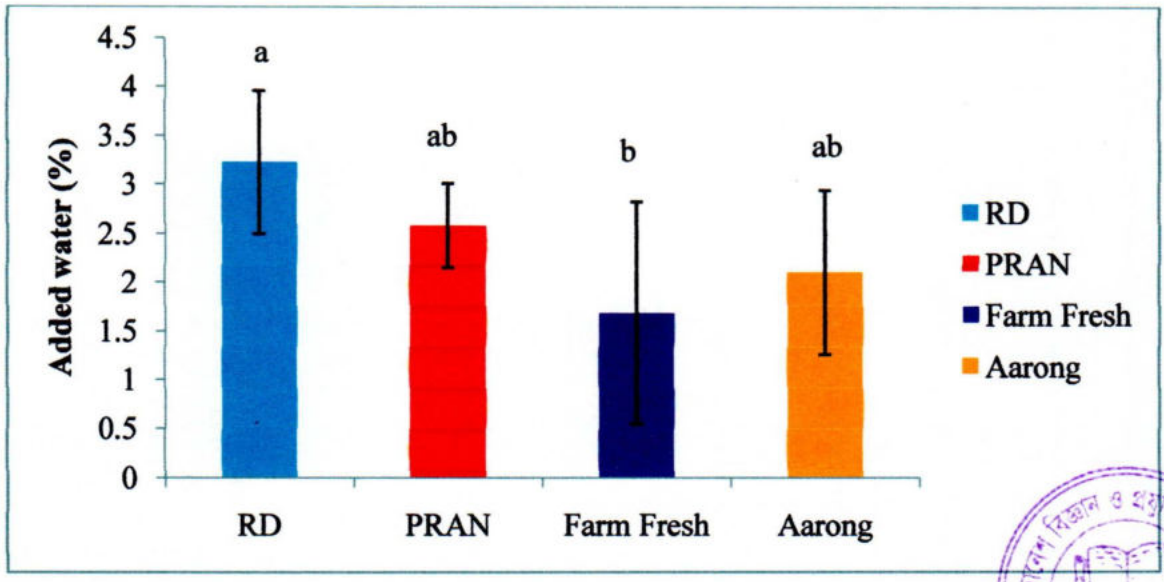

Figure 4.6: Added water content in different UHT milk.

4.2.2 Hydrogen Peroxide.

Table 4.3 revealed that the examined UHT milk samples were negative for hydrogen peroxide. Negative results were reported by Abdel-Hameid (2002), Wahba and Korashy (2006). Corresponding results for dairy shops, 2 (3.3%) out of 60 examined cow's milk samples were positive. But higher results were recorded by El-Bessary (2006), whereas Wahba and Korashy (2006) reported negative results. Faraz *et al.* (2013) was reported that the hydrogen peroxide adulteration found in 3% raw milk samples (60 samples for 6 weeks collection) from various canteens of public places.

4.2.3 Soda (Rosolic acid)

Table 4.3 revealed that the examined UHT milk samples were negative for soda test. Negative results represented that the UHT milk did not adulterated by soda. Sodium carbonate or sodium bicarbonate is sometimes added to milk to reduce the acidity of milk (lactic acid formed as a result of lactic fermentation).

4.2.4 Formaldehyde

Formalin is a famous preservative for milk because it has the property of being in liquid form. Table 4.3 indicated that all examined milk samples of UHT milk were free from formalin. Similar data were reported by Moustafa (1978), El-Bessary (2006), while Kamel (2000) was found that 30% of raw market milk samples were positive. Formalin adulteration was present in 23% and 27% raw milk samples (60 samples for 6 weeks collection) from various canteens of educational institutes and public places, respectively (Faraz *et al.*, 2013).

4.2.5 Starch

Table 4.3 indicated that all examined milk samples of UHT milk were free from starch. Milk contains relatively large amount of fat. Addition of carbohydrate to milk increases its solid content. There by reducing the amount of fat present in the milk. Starch is one such component that is added to adulterate milk (amrita.vlab.co.in). Faraz *et al.* (2013) was reported that the raw milk samples collected from canteens of educational institutes and public places were free from addition of starch out of 60 samples which was collected for at least 6 weeks.

4.2.6 Cane Sugar

Table 4.3 indicated that all examined milk samples of UHT milk were free from cane sugar. The common sugar present in milk is lactose. The fat content of the milk is more compared to the protein content. Cane sugar like sucrose is added to the milk to increase the carbohydrate content of the milk and thus the density of milk will be increased (amrita.vlab.co.in). Faraz *et al.* (2013) was found that 87% and 97% raw milk samples (60 samples for 6 weeks collection) showed cane sugar adulteration from various canteens of educational institutes and public places, respectively.

4.2.7 Carbonates

All examined UHT milk samples collected from local market were free from carbonate and bi-carbonate (Table 4.3). Ibrahim *et al.*, (2012), found that the cow's dairy shops examined milk samples, 3 (5%) out of 60 examined milk samples were positive. On the other hand, street vendors examined milk samples, 4 (6.7%) out of 60 examined raw milk samples were positive for carbonate and bi-carbonate.

4.2.8 Skim Milk Powder

All examined UHT milk samples collected from local market were free from skim milk powder (Table 4.3).

4.2.9 Sodium Chloride

All examined UHT milk samples collected from local market were free from sodium chloride (Table 4.3). Sometimes sodium chloride is added to milk to increase the pH value thus lowering the titratable acidity.

4.2.10 Pulverized Soap

All examined UHT milk samples which was collected from local market were free from pulverized Soap (Table 4.3). Pulverized soap was added to milk to increase the foaming of milk and thus to have thick milk. Addition of such chemicals will cause health problem especially related to stomach and kidneys.

CHAPTER V

SUMMARY AND CONCLUSION

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The study was conducted in the laboratory of the Department of Food processing and Preservation and the Department of Dairy and poultry science, Hajee Mohammad Danesh Science and Technology University, Dinajpur, to determination of physic-chemical parameters and adulteration of UHT milk available in Bangladesh.

Protein content of UHT milk obtained from RD, PRAN, Farm Fresh and Aarong was 3.372%, 3.202%, 3.417% and 3.258%, respectively. But each UHT milk processing company labeled the nutrition content into the package and the protein content is 3.4%. Only one UHT milk processing company (Farm Fresh) maintained that standard.

Fat percentage of UHT milk obtained from RD, PRAN, Farm Fresh and Aarong was 3.133, 3.40, 3.50 and 3.5, respectively. The statistical analysis showed that the fat content of Farm Fresh and Aarong UHT milk was higher than other samples (RD and PRAN).

Titratable acidity of UHT milk obtained from RD, PRAN, Farm Fresh and Aarong was 0.1717, 0.1917, 0.1833 and 0.1833, percentages respectively. All of the tested UHT milk samples titratable acidity was higher than the standard acidity (0.15%).

pH of UHT milk obtained from RD, PRAN, Farm Fresh and Aarong was 6.30, 6.10, 6.133 and 6.217, respectively. The tested samples pH was lower than the standard pH (6.4 to 6.6).

Corrected lactometer reading of UHT milk obtained from RD, PRAN, Farm Fresh and Aarong was 27.90, 27.87, 28.10 and 27.97, respectively.

Specific gravity of UHT milk obtained from RD, PRAN, Farm Fresh and Aarong was 1.0279, 1.0278, 1.0281 and 1.0279, respectively.

Solids-not-fat (SNF) of UHT milk obtained from RD, PRAN, Farm Fresh and Aarong was 7.742%, 7.793%, 7.865% and 7.832%, respectively. All of the tested UHT milk samples SNF was lower than the standard SNF (8.0%).

Total solids (TS) of UHT milk obtained from RD, PRAN, Farm Fresh and Aarong was 10.88%, 11.31%, 11.36% and 11.33%, respectively.

Water content of UHT milk obtained from RD, PRAN, Farm Fresh and Aarong was 89.13%, 88.69%, 88.64% and 88.67%, respectively.

The clot on boiling and alcohol test for the different UHT milk showed the negative result.

Added water content of UHT milk obtained from RD, PRAN, Farm Fresh and Aarong was 3.229%, 2.583%, 1.688% and 2.104%, respectively. The examined UHT milk samples were negative for all adulteration tests i.e. hydrogen peroxide, rosolic acid, formaldehyde, starch, cane sugar, carbonates, skim milk powder, sodium chloride and pulverized soap (except added water).

Milk is almost a complete food which contains most of the proximate composition of a balanced diet and this is why milk is highly sensitive to be contaminated by bacteria. But consumer prefers wholesome and nutritious food product that means the product is free from pathogens and maintained the standard of composition. For this reason, milk is processed by UHT with integrated aseptic packaging to remove microorganisms and to increase the shelf life of milk.

Upon this study, it can be concluded that the different milk processing companies do not maintain all the BSTI (2002) standards. The statistical analysis showed that Farm Fresh UHT milk was more acceptable as compared to RD, PRAN and Aarong milk. Finally, the legal authority should be aware of this fact and must maintain the rules and regulations for the standard of milk.


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APPENDICES

APPENDICES

Appendix 1 Pasteurized Milk Standard* (BSTI, 2002)

Parameters	Standard	Parameters	Standard
Fat	3.5%	SNF	8.00%
Protein	minimum 3.3%	CLR	28.0
pH	6.4- 6.6	Specific gravity	1.028
Titratable acidity	0.15%	Water content	84.0- 88.0%
TS (Total solids)	12.0%		

*Note: The above standards are followed by the dairy industries for UHT milk in Bangladesh.

Appendix 2 Six Independent Data for Protein Content (%)

Sl No	RD	PRAN	Farm Fresh	Aarong
1	3.40	3.06	3.57	3.23
2	3.57	3.23	3.40	3.40
3	3.40	3.06	3.57	3.06
4	3.23	3.23	3.40	3.23
5	3.40	3.23	3.23	3.40
6	3.23	3.40	3.40	3.23

Appendix 3 Analysis of Variance (ANOVA) for Protein Content

Source	Degree of freedom	Sum of squares	Mean squares	F- value	
				Calculated	Tabulated
Sample	4	0.194	0.048	3.31	2.97
Replication	5	0.035	0.007	0.47	2.71
Error	20	0.293	0.015		
Total	29	0.521			

Appendix 4 Duncan's Multiple Range Test (DMRT) for Protein Content

LSD value = 0.1475, P<0.05

Sample	Original order of means	Sample	Ranked order of means
RD	3.372 ± 0.13^{ab}	Farm Fresh	3.417 ± 0.13^{a}
PRAN	3.202 ± 0.13^{c}	RD	3.372 ± 0.13^{ab}
Farm Fresh	3.417 ± 0.13^{a}	Standard	3.30 ± 0.00^{abc}
Aarong	3.258 ± 0.13^{bc}	Aarong	3.258 ± 0.13^{bc}
Standard	3.30 ± 0.00^{abc}	PRAN	3.202 ± 0.13^{c}

Appendix 5 Six Independent Data for Fat Content (%)

Sl No	RD	PRAN	Farm Fesh	Aarong
1	3.2	3.2	3.6	3.4
2	3.1	3.2	3.4	3.6
3	3.2	3.5	3.5	3.6
4	3.1	3.5	3.5	3.5
5	3.2	3.6	3.4	3.4
6	3.0	3.4	3.6	3.5

Appendix 6 Analysis of Variance (ANOVA) for Fat Content

Source	Degree of freedom	Sum of squares	Mean squares	F- value	
				Calculated	Tabulated
Sample	4	0.605	0.151	13.5928	2.97
Replication	5	0.031	0.006	0.5509	2.71
Error	20	0.223	0.011		
Total	29	0.859			

Appendix 7 Duncan's Multiple Range Test (DMRT) for Fat Content
LSD value = 0.1263, P<0.05

Sample	Original order of means	Sample	Ranked order of means
RD	3.133 ± 0.08^{b}	Farm Fresh	3.500 ± 0.09^{a}
PRAN	3.400 ± 0.17^{a}	Aarong	3.500 ± 0.09^{a}
Farm Fresh	3.500 ± 0.09^{a}	Standard	3.500 ± 0.00^{a}
Aarong	3.500 ± 0.09^{a}	PRAN	3.400 ± 0.17^{a}
Standard	3.500 ± 0.00^{a}	RD	3.133 ± 0.08^{b}

Appendix 8 Six Independent Data for pH Content

Sl No	RD	PRAN	Farm Fresh	Aarong
1	6.3	6.1	6.2	6.3
2	6.2	6.0	6.3	6.2
3	6.3	6.2	6.1	6.1
4	6.2	6.0	6.0	6.2
5	6.4	6.1	6.1	6.3
6	6.4	6.2	6.1	6.2

Appendix 9 Analysis of Variance (ANOVA) for pH Content

Source	Degree of freedom	Sum of squares	Mean squares	F- value	
				Calculated	Tabulated
Sample	4	0.613	0.153	25.00	2.97
Replication	5	0.039	0.008	1.2717	2.71
Error	20	0.123	0.006		
Total	29	0.775			

Appendix 10 Duncan's Multiple Range Test (DMRT) for pH Content

LSD value = 0.09329, P<0.05

Sample	Original order of means	Sample	Ranked order of means
RD	6.300 ± 0.09^{b}	Standard	6.50 ± 0.00^{a}
PRAN	6.100 ± 0.09^{d}	RD	6.300 ± 0.09^{b}
Farm Fresh	6.133 ± 0.10^{cd}	Aarong	6.217 ± 0.08^{bc}
Aarong	6.217 ± 0.08^{bc}	Farm Fresh	6.133 ± 0.10^{cd}
Standard	6.50 ± 0.00^{a}	PRAN	6.100 ± 0.09^{d}

Appendix 11 Six Independent Data for Water Content (%)

Sl No	RD	PRAN	Farm Fresh	Aarong
1	89.07	89.02	88.44	88.83
2	89.24	88.97	88.63	88.64
3	89.02	88.66	88.61	88.59
4	89.09	88.21	88.66	88.66
5	89.02	88.64	88.83	88.73
6	89.31	88.64	88.64	88.56

Appendix 12 Analysis of Variance (ANOVA) for Water Content

Source	Degree of freedom	Sum of squares	Mean squares	F- value	
				Calculated	Tabulated
Sample	4	38.049	9.512	364.2791	2.97
Replication	5	0.101	0.020	0.7746	2.71
Error	20	0.522	0.026		
Total	29	38.672			

Appendix 13 Duncan's Multiple Range Test (DMRT) for Water Content

LSD value = 0.1942, P<0.05

Sample	Original order of means	Sample	Ranked order of means
RD	89.13±0.12[a]	RD	89.13±0.12[a]
PRAN	88.69±0.29[b]	PRAN	88.69±0.29[b]
Farm Fresh	88.64±0.12[b]	Aarong	88.67±0.10[b]
Aarong	88.67±0.10[b]	Farm Fresh	88.64±0.12[b]
Standard	86.00±0.00[c]	Standard	86.00±0.00[c]

Appendix 14 Six Independent Data for Solids Not Fat (SNF)

Sl No	RD	PRAN	Farm Fresh	Aarong
1	7.73	7.78	7.96	7.77
2	7.66	7.83	7.97	7.76
3	7.78	7.84	7.89	7.81
4	7.81	7.79	7.84	7.84
5	7.78	7.76	7.77	7.87
6	7.69	7.76	7.76	7.94

Appendix 15 Analysis of Variance (ANOVA) for Solids Not Fat (SNF)

Source	Degree of freedom	Sum of squares	Mean squares	F- value	
				Calculated	Tabulated
Sample	4	0.228	0.057	13.6950	2.97
Replication	5	0.004	0.001	0.1895	2.71
Error	20	0.083	0.004		
Total	29	0.315			

Appendix 16 Duncan's Multiple Range Test (DMRT) for Solids Not Fat (SNF)

LSD value = 0.07617, P<0.05

Sample	Original order of means	Sample	Ranked order of means
RD	7.742 ± 0.06^{c}	Standard	8.00 ± 0.0^{a}
PRAN	7.793 ± 0.03^{b}	Farm Fresh	7.865 ± 0.09^{a}
Farm Fresh	7.865 ± 0.09^{a}	Aarong	7.832 ± 0.07^{ab}
Aarong	7.832 ± 0.07^{ab}	PRAN	7.793 ± 0.03^{b}
Standard	8.00 ± 0.0^{a}	RD	7.742 ± 0.06^{c}

Appendix 17 Six Independent Data for Titratable Acidity

Sl No	RD	PRAN	Farm Fesh	Aarong
1	6.3	6.1	6.2	6.3
2	6.2	6.0	6.3	6.2
3	6.3	6.2	6.1	6.1
4	6.2	6.0	6.0	6.2
5	6.4	6.1	6.1	6.3
6	6.4	6.2	6.1	6.2

Appendix 18 Six Independent Data for Corrected Lactometer Reading (CLR)

Sl No	RD	PRAN	Farm Fresh	Aarong
1	27.8	28.0	28.4	27.8
2	27.6	28.2	28.6	27.6
3	28.0	28.0	28.2	27.8
4	28.2	27.8	28.0	28.0
5	28.0	27.6	27.8	28.2
6	27.8	27.6	27.6	28.4

Appendix 19 Analysis of Variance (ANOVA) for Corrected Lactometer Reading (CLR)

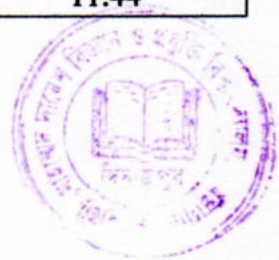
Source	Degree of freedom	Sum of squares	Mean squares	F- value	
				Calculated	Tabulated
Sample	4	0.200	0.050	0.6345	2.97
Replication	5	0.071	0.014	0.1794	2.71
Error	20	1.576	0.079		
Total	29	1.847			

Appendix 20 Six Independent Data for Specific Gravity (Sp. Gr.)

Sl No	RD	PRAN	Farm Fresh	Aarong
1	1.0278	1.028	1.0284	1.0278
2	1.0276	1.0282	1.0286	1.0276
3	1.028	1.028	1.0282	1.0278
4	1.0282	1.0278	1.028	1.028
5	1.028	1.0276	1.0278	1.0282
6	1.0278	1.0276	1.0276	1.0284

Appendix 21 Six Independent Data for Total Solids (TS)

Sl No	RD	PRAN	Farm Fresh	Aarong
1	10.93	10.98	11.56	11.17
2	10.76	11.03	11.37	11.36
3	10.98	11.34	11.39	11.41
4	10.91	11.79	11.34	11.34
5	10.98	11.36	11.17	11.27
6	10.69	11.36	11.36	11.44



Appendix 22 Analysis of Variance (ANOVA) for Total Solids (TS)

Source	Degree of freedom	Sum of squares	Mean squares	F- value	
				Calculated	Tabulated
Sample	4	3.881	0.970	37.1559	2.97
Replication	5	0.101	0.020	0.7746	2.71
Error	20	0.522	0.026		
Total	29	4.504			

Appendix 23 Duncan's Multiple Range Test (DMRT) for Total Solids (TS)

LSD value = 0.1942, P<0.05

Sample	Original order of means	Sample	Ranked order of means
RD	10.88 ± 0.12^{c}	Standard	12.00 ± 0.00^{a}
PRAN	11.31 ± 0.29^{b}	Farm Fresh	11.36 ± 0.12^{b}
Farm Fresh	11.36 ± 0.12^{b}	Aarong	11.33 ± 0.10^{b}
Aarong	11.33 ± 0.10^{b}	PRAN	11.31 ± 0.29^{b}
Standard	12.00 ± 0.00^{a}	RD	10.88 ± 0.12^{c}

Appendix 24 Six Independent Data for Percentage of Added Water Content

Sl No	RD	PRAN	Farm Fresh	Aarong
1	3.375	2.75	0.5	2.875
2	4.25	2.125	0.375	3.00
3	2.75	2.00	1.375	2.375
4	2.375	2.625	2.00	2.00
5	2.75	3.00	2.875	1.625
6	3.875	3.00	3.00	0.75

Appendix 25 Analysis of Variance (ANOVA) for Percentage of Added Water Content

Source	Degree of freedom	Sum of squares	Mean squares	F- value	
				Calculated	Tabulated
Sample	3	7.898	2.633	3.3772	3.28
Replication	5	0.769	0.154	0.1797	2.90
Error	15	12.833	0.856		
Total	23	21.499			

Appendix 26 Duncan's Multiple Range Test (DMRT) for Percentage of Added Water Content

LSD value = 1.247, P<0.05

Sample	Original order of means	Sample	Ranked order of means
RD	3.229 ± 0.073^{a}	RD	3.229 ± 0.073^{a}
PRAN	2.583 ± 0.43^{ab}	PRAN	2.583 ± 0.43^{ab}
Farm Fresh	1.688 ± 1.14^{b}	Aarong	2.104 ± 0.84^{ab}
Aarong	2.104 ± 0.84^{ab}	Farm Fresh	1.688 ± 1.14^{b}