RESEARCH PAPER
Research Journal of Animal Husbandry and Dairy Science ⇨ ISSN-0976-5646
Volume 10 | Issue 1 | June, 2019 | 1-13 ■ DOI: 10.15740/HAS/RJAHDS/10.1/1-13
Visit us: *www.researchjournal.co.in*

Study of chemical qualities of raw buffalo milk at different milking times

U.K. Shukla and Arvind Shukla

ABSTRACT : To study of chemical qualities of raw milk of buffalo was conducted at chitrakoot satna (M .P.) the objective was to find out the chemical quality of buffalo's on fat, protein, lactose ash water, sp, gr., S.N.F. and T. S. compositional quality of raw milk and under full hand diagonal method of milking at mini dairy farm statistical analysis applying the technique of analysis of variance (f-test) the most widely used method for determining protein content by kjeldahi method for nitrogen determination since nitrogen is a characteristic element in protein by its accurate determination protein characteristic can be finding animal showed considerable variation regarding the principal components in milk. Results presented above, that the chemical quality of raw buffalo milk at morning (M_1) was found best in terms of maximum protein (%), specific gravity (cc), fat (%), lactose (%), total solid (%) and solid not fat (SNF) (%) and minimum ash (%) and water (%); followed by evening milk (M_3) and noon milk (M_2).

KEY WORDS : Raw milk, Chemical quality, Buffalo milking, Times

HOW TO CITE THIS PAPER : Shukla, U.K. and Shukla, Arvind (2019). Study of chemical qualities of raw buffalo milk at different milking times. *Res. J. Animal Hus. & Dairy Sci.,* **10**(1) : 1-13 : **DOI: 10.15740/HAS/RJAHDS/10.1/1-13.**

INTRODUCTION

Milk- milk may be defined as "the whole, fresh, clean, lacteal secretion practically free from colostrum obtained by the complete milking of one or more healthy milch animals, which contains not less than 8.25 per cent milk solids not fats and not less than 3.25 per cent milk fat (Atherton and Newlander, 1987).

Chemical composition of milk- milk and milk products are main constituents of the diet, especially for vulnerable groups such as infants, school age children and old age (Davies *et al.,* 1986). Fresh milk is neutral or slightly alkaline but on souring becomes acidic because of the lactic acid formed by bacterial action on lactose.

MEMBERS OF RESEARCH FORUM

Address for correspondence :
U.K. Shukla, Department of Natural Resource Management, Faculty of Agriculture, Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya, Chitrakoot, **Satna (M.P.) India**
Email : umeshdr2006@rediffmail.com

Associated Authors' :
Arvind Shukla, Livestock Production and Management (unit), Department of Natural Resource Management, Faculty of Agriculture, Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya, Chitrakoot, **Satna (M.P.) India**
Email : arvindas7020@gmail.com

Major milk constituents:

Water constitutes the medium in which the other milk constituents are either dissolved or suspended. Small portion of this water is firmly bound to milk proteins, phospholipids etc. while major amount of it is in a free form. This free water is most essential for growth and activities of micro-organisms. The second major constituent of milk is lactose sugar which serves as a major carbon source for majority of spoilage causing organisms. It exists only in milk in a true solution form and is responsible for slight sweet taste of milk. It occurs in α and β forms, both of which occur as the hydrate or the anhydride. Mainly lactic acid and other organic acids

are produced by bacteria due to fermentation of lactose. This fermentation activity is important in the production of cultured milk products and spoilage of milk and milk products generally by souring. Approximately 3.4 per cent is the total fat content of milk which plays a significant role in determining the physical properties, nutritive value and flavour of milk and milk products. It has been detected that milk fat contains more than 400 different fatty acids of which approximately 15-20 fatty acids are involved in makeup of 90 per cent milk fat. Straight chain fatty acids like saturated fatty acids (65%) which have 4 to 18 carbons, monounsaturated fatty acids (30%) and polyunsaturated fatty acids (5%) are the major fatty acids in milk fat. The fatty acids are arranged in specific manner on the triglyceride molecule in which most of the short chain fatty acids are at the bottom carbon position and longer fatty acids tend to be in the middle and top positions of the triglyceride molecule. This distribution of fatty acids on the triglyceride affects flavor, nutritional properties and physical properties of milk fat like hydrophobicity, density, melting characteristics etc. The triglycerides which constitute 98 per cent of the milk fat are in the form of globules which are surrounded by a protein and phospholipids membrane which stabilizes globules in serum phase of milk. The size of native globules varies from less than 1 µm to more than 10 µm. In addition to triglycerides, milk also contains other lipids like cholesterol, diglycerides, free fatty acids, phospholipids and cerebrosides. A forth important major constituent of the milk is the proteins. The total protein content of milk is approximately 3.3 per cent which is a rich source of all nine essential amino acids required by a human being with a high nutritional value (Harding, 1999). Based 3 on the chemical composition and physical properties, the milk proteins are defined into two major categories. The major proteins in cow's milk are the caseins which have approximately 82 per cent of milk protein and have three subclasses such as α-casein, β-casein and κ-casein. Each subclass of the caseins has its own amino acid composition, genetic variation and functional properties. The caseins coagulate or precipitate at pH 4.6 and contains phosphorus. The second category of milk proteins is serum (whey) proteins which always remain in solution in milk at pH 4.6 and do not contain any phosphorus but do contain in a large amounts of sulfur containing amino acids. Serum or whey proteins constitutes 18 per cent of milk proteins which includes α- lactoalbumin (20%) and β–lactoglobulin (50%) (Yadav *et al.*, 1993). Each serum protein has its own characteristic composition and variations. The functions of many whey proteins are not clearly defined but that of β - lactoglobulin is thought to be a carrier of vitamin A and α–lactoalbumin plays important role in lactose synthesis in a memory gland. Lactoferrin and transferrin plays role in iron absorption. Caseins are highly digestable in intestine while whey proteins are relatively less digestable in intestine.

Minor milk constituents:

There are three types of phospholipids in milk, cephalin, lecithin and sphingomylin. Lecithin contributes to the flavour to milk and other dairy products. Phospholipids stabilize the milk fat emulsion as it is a excellent emulsifying agent. Cholesterol present in fat solution and the part of the fat globule membrane. The second minor constituent of milk includes fat soluble and water soluble pigments. Fat soluble pigments includes carotene and xanthophylls and water soluble pigment includes riboflavin. The important milk enzymes are lipase, amylase, protease, phosphatase, catalase and peroxidase. More than 25 vitamins have been reported to be present in milk most of which cannot be produced within the body and, therefore, need to be provided in the diet (few miligrams or micrograms per day). Vitamin D can be obtained by the action of sunlight on the skin and B vitamin can be made from tryptophan amino acids. Fat soluble vitamins includes vitamin A (Retinol and β-carotene), vitamin D (Calciferol), vitamin E (Tocopherol) and vitamin K. Water soluble vitamins includes B complex vitamins, e.g. Thiamine (B_1), Riboflavin (B_2), Niacin (B_3), etc. and small quantities of vitamin C (ascorbic acid) is present in milk which can be destroyed by souring, oxidation and heat (Burdova *et al*., 2002). 4 Many trace elements that are essential for growth are present in milk that includes calcium, sodium, potassium, phosphorus, zinc, cobalt, iodine, iron etc (Stewart, 1978 and Harding, 1999). The colour of milk is largely due to the presence of carotene (α–carotene, β-carotene and γ-carotene) as it is found in hay, grass and green leaves (Salle, 1974). Another pigment closely related to carotene is cryptoxanthin which occurs in yellow colour. The pH of normal milk usually varies from 6.4 to 6.6 for cow milk and for buffalo milk varies from 6.7 to 6.8.

Buffalo milk has higher levels of fat, lactose, protein, ash and Ca, and vitamins A and C and lower levels of

vitamin E, riboflavin and cholesterol; an absence of carotene and the presence of the blue-green pigment (biliverdin) as well as a bioactive pentasaccharide and gangliosides, the fat has slightly higher levels of saturated fatty acids and has quantitative differences in the distribution of triglycerides and physical properties, the casein micelles of buffalo milk are larger and richer in minerals, the primary structures of all buffalo milk proteins have been established. Buffalo milk α-casein and β-casein have lower levels of phosphorylation, the viscosity and curd tension of buffalo milk are higher; rennet coagulation is faster and heat stability is lower than that of cow milk (Abd El-Salam and El-Shibiny, 2011).

Buffalo milk like cow milk is composed of similar constituents but distinctly differs in the lovely of these constituents (Shukla and Patel, 2017).

Milk plays a tremendous role in building a healthy society and can be used as a vehicle for rural development, employment and slowing down the migration of the rural population (Sarwar *et al*., 2002). It is ideal for microbial growth and the fresh milk easily deteriorates to become unsuitable for processing and human consumption (Dehinenet *et al.*, 2013). But milk adulteration is a common phenomenon, especially in certain areas of the world where water, starch solution, industrial alkalies and nitrites are common materials added in milk. Milk adulteration leads to economic losses, deterioration of the quality of the end products and a risk to the consumers safety. The most common form of milk adulteration has been by adding water to milk which may be polluted sswith faeces, micro-organisms, harmful chemicals and poisonous substances. So the milk will be contaminated with those substances. Also addition of water decrease the milk SNF (Solids Not Fat) contents especially proteins which are very important for normal growth. Furthermore another important type of adulteration is the removal of fat. Skimming of fat inhibit the body from utilization of fat, fat soluble vitamins as A,D,E and K which are very important for biological processes and normal growth of the body (Kartheek *et al*., 2011).

Milk has been recognized as a vegetarian food since ancient times in India and all Indians consume milk and milk products without reticence. According to Prevention of Food Adulteration Act (PFA) Rules (1955), milk is the normal mammary secretion, free from colostrum, derived from complete milking of healthy milk animal. To increase the shelf-life and organoleptic properties of milk and milk products are the two main objectives of the dairy industries. Milk and milk products form a significant part of the human diet. It has a wide range of positive nutritional benefits and supplies a variety of nutrients including protein for bodybuilding, vitamins, minerals (especially calcium), fat and carbohydrate for energy (Harding, 1999).

MATERIAL AND METHODS

Collection of raw milk samples:

Estimation of protein:

The most widely used method for determining protein content is by Kjeldahl method for nitrogen determination. Since nitrogen is a characteristic element in protein, by its accurate determination, protein concentration can be calculated. In 1883, Johann Kjeldahl developed the basic procedure to analyze organic nitrogen. The method involves two major steps. In the first step, the protein is digested using concentrated sulphuric acid in presence of a catalyst. In this step all the organic material is oxidized except nitrogen, the reduced form of which is retained in digest as ammonium sulphate. Neutral salts such as potassium sulphate are used in the digestion step to raise the boiling point of the reaction mixture and thereby effectively increase the digestion rate. Metallic catalyst such as copper sulfate is used to hasten the digestion and clearing the reaction mixture. The digest is neutralized with alkali to liberate ammonia.

In the second step, ammonia is distilled off, collected in boric acid and titrated with standard acid. Boric acid provides the most convenient absorbent for ammonia in that, the need for a standard alkali in titration is eliminated, and neither the amount nor the concentration of boric acid needs to be precise, since the boric acid itself is not involved in the titration, but simply reacts with the ammonia to form an ammonium borate complex. The strongly basic ammonium-borate that is formed is titrated directly with acid in the presence of a methyl red-bromocresol green indicator until the green distillate changes through colourless to pink.

Add to the clean and dry Kjeldahl flask, 5 – 10 boiling aids, 15 g K_2SO_4, 1.0 ml of the copper sulfate solution, approximately 5 ml of milk sample and add 25 ml of concentrated sulfuric acid. Use the 25 ml acid also to wash down any copper sulfate solution, K_2SO_4 or milk left on the neck of the flask. Gently mix the contents of the Kjeldahl flask. Turn on the fume extraction system

of the digestion apparatus prior to beginning the digestion. Heat the Kjeldahl flask and its contents on the digestion apparatus using a heater setting low enough such that charred digest does not foam up the neck of the Kjeldahl flask. Digest at this heat-setting for at least 20 min or until white fumes appear in the flask. Increase the heater setting to half way to the and continue the heating period for 15 min. At the end of 15 min period, increase the heat to maximum. After the digest clears (clear with light blue-green colour), continue boiling for 1 h to 1.5 h at maximum setting. The total digestion time will be between 1.8 – 2.25 h.

At the end of digestion, the digest shall be clear and free of undigested material. Allow the acid digest to cool to room temperature over a period of approximately 25 min. If the flasks are left on hot burners to cool, it will take longer to reach room temperature. The cooled digest should be liquid or liquid with a few small crystals at the bottom of the flask at the end of 25 min cooling period. Do not leave the undiluted digest in the flask overnight. The undiluted digest feb.to march crystallize during this period and it will be very difficult to get that back into the solution to avoid this situation.

After the digest is cooled to room temperature, add 300 ml of water to 500 ml Kjeldahl flask or 400 ml of water when using 800 ml Kjeldahl flask. Use the water to wash down the neck of the flask too. Mix the contents thoroughly ensuring that any crystals which separate out are dissolved. Add 5 - 10 boiling aids. Allow the mixture to cool again to room temperature prior to the distillation. Diluted digests feb.to march be stoppered and held for distillation at a later time.

Titrate the boric acid receiving solution with standard hydrochloric acid solution (0.1 N) to the first trace of pink colour. Take the burette reading to at least the nearest 0.05 ml. A lighted stir plate feb.to march aid visualization of the end point.

$$W_n = \frac{1.4007 \times (V_s - V_B) \times N}{W}$$

where,

W_n = Nitrogen content of sample, expressed as a percentage by mass.

V_S = Volume in ml of the standard hydrochloric acid used for sample.

V_B = Volume in ml of the standard hydrochloric acid used for blank test.

N = Normality of the standard hydrochloric acid expressed to four decimal places.

W = Mass of test portion in g, expressed to nearest 0.1 mg.

Estimation of specific gravity:

Measure 10 ml of sulphuric acid into a butyro meter tube, preferably by use of an automatic dispenser, without wetting the neck of the tube. Mix the milk sample gently but thoroughly and fill the milk pipette above the graduation line. Wipe the outside of the pipette and allow the milk level to fall so that the top of meniscus is level with the mark. Run the milk into the butyrometer tube along the side wall without wetting the neck, leave to drain for three seconds and touch the pipette's tip once against the base of the neck of the butyrometer tube. Add 1 ml of Amyl alcohol, close with a lock stopper, shake until homogeneous, inverting it for complete admixture of the acid. Keep in a water bath for 5 min. at 65±2°C taking care to have casein particles if any to dissolve fully and centrifuge for 4 min. at 1100 rpm. The tubes should be put in centrifuge, so as to conform to radial symmetry, and as evenly spaced as possible, in order to protect bearings of the centrifuge. Allow the centrifuge to come to rest. Remove the butyrometer tubes and place in water bath for 5 min. at 65±2°C. Read the percentage of fat after adjusting the height in the tube as necessary by movements of the lock stopper with the key. Note the scale reading corresponding to the lowest point of the fat meniscus and the surface of separation of the fat and acid. When readings are being taken hold the butyrometer with the graduated portion vertical, keep the point being read in level with the eye, and then read the butyrometer to the nearest half of the smallest scale division.

Estimation of fat:

The milk is mixed with sulphuric acid and iso-amyl alcohol in a special Gerber tube, permitting dissolution of the protein and release of fat. The tubes are centrifuged and the fat rising into the calibrated part of the tube is measured as a percentage of the fat content of the milk sample. The method is suitable as a routine or screening test. It is an empirical method and reproducible results can be obtained if procedure is followed correctly.

Measure 10 ml of sulphuric acid into a butyrometer tube, preferably by use of an automatic dispenser, without wetting the neck of the tube. Mix the milk sample gently but thoroughly and fill the milk pipette above the graduation

line. Wipe the outside of the pipette and allow the milk level to fall so that the top of meniscus is level with the mark. Run the milk into the butyrometer tube along the side wall without wetting the neck, leave to drain for three seconds and touch the pipette's tip once against the base of the neck of the butyrometer tube. Add 1 ml of Amyl alcohol, close with a lock stopper, shake until homogeneous, inverting it for complete admixture of the acid. Keep in a water bath for 5 min. at 65±2°C taking care to have casein particles if any to dissolve fully, and centrifuge for 4 min. at 1100 rpm. The tubes should be put in centrifuge, so as to conform to radial symmetry, and as evenly spaced as possible, in order to protect bearings of the centrifuge. Allow the centrifuge to come to rest. Remove the butyrometer tubes and place in water bath for 5 min. at 65±2°C. Read the percentage of fat after adjusting the height in the tube as necessary by movements of the lock stopper with the key. Note the scale reading corresponding to the lowest point of the fat meniscus and the surface of separation of the fat and acid. When readings are being taken hold the butyrometer with the graduated portion vertical, keep the point being read in level with the eye and then read the butyrometer to the nearest half of the smallest scale division.

The fat can be calculated using following formula:

$$\text{Fat \% (w/w)} = \frac{\text{Weight of extracted fat}}{\text{Fat weight of milk}} \times 100$$

Estimation of lactose:

This method is useful in distinguishing milk sweets prepared from *Khoa* and *Paneer* or *Channa*. The above method is based upon the reaction of lactose with methylamine in hot alkaline solution to form a red complex which absorbs at 540 nm. The method is useful for differentiating *Khoa* based and *Chhanna* based sweets.

To 8.0 g of well mixed sample add 1 ml of ZAPT reagent, dilute to 10 ml, and after 10 minutes filter using Whatman No. 1 filter paper, to 0.5 ml of the filtrate add 0.5 ml of NaOH solution, dilute to 10 ml and filter using Whatman No. 1 filter paper. Dilute 5 ml of the filtrate to 10 ml.

Pipette 5 ml each of working standard lactose and unknown solution into 25 ml test tubes. Add 5 ml of glycine NaOH buffer, 0.5 ml of methylamine solution and 0.5 ml of sodium sulphite solution in each tube, mix thoroughly. Heat tubes in a thermostatically controlled water bath at 65°C for 25 min. and cool immediately in an ice water bath for 2 min. to stop the reaction. Read absorbance against blank at 540nm in a spectrophotometer or a suitable spectrophotometer. Draw a standard curve by plotting absorbance against concentration of lactose and determine the concentration of lactose from it.

Estimation of ash:

Total nitrogen content in milk powder sample is estimated by Kjeldahl method as described for milk. The per cent nitrogen obtained is multiplied by a factor to get protein content in milk powder sample.

The ash value can be calculated by following formula:

Calculate crude protein content = N x 6.38

where,

N = Nitrogen content in sample estimated by Kjeldahl method.

Determination of total solids:

In this procedure, a known quantity of milk is dried on a boiling water bath. Subsequently sample is dried in hot air oven at 102 ±2°C and from the weight of the residue, the total solids content in milk is determined.

Transfer sample to a beaker, warm slowly to 35° - 40°C on a water bath with careful mixing to incorporate any cream adhering to the sample. Cool the sample quickly to room temperature. Heat a dish with its lid alongside in the drying oven at least 1 hour. Place the lid on the dish and immediately transfer to a desiccator. Allow cooling to room temperature (at least 30 mins) and weighing to the nearest 0.1 mg. Add 5 ml of prepared sample, place the lid on the dish and weigh again. Place the dish without the lid on the vigorously boiling water bath in such a way that the bottom of the dish is directly heated by the steam. Continue heating till most of the water is removed. Remove the dish from the water bath, wipe the underside and place it in the oven alongside the lid and dry in the oven for 2 hours. Place the lid and transfer to the desiccators. Allow the dish to cool and weigh to the nearest 0.1 mg. Again heat the dish with its lid alongside in the oven for 1 hour. Place the lid on the dish and immediately transfer to the desiccators. Allow to cool and weigh again. Repeat the operation again until the difference in the two consecutive weighing does not exceed 1 mg. Record the lowest mass.

The total solid can be determine using following formula:

$$\text{Total solid content} = \frac{M_2 - M_0}{M_1 - M_0} \times 100$$

where,
M_0 = mass in g of dish + lid
M_1 = mass in g of dish + lid and test portion
M_2 = mass in g of dish + lid and dried test portion
Round the value obtained to nearest 0.01 % (m/m)

Determination of water:

Water per cent
Water per cent = 100-T.S.
Wher, T.S. = Total soilds

Statistical analysis of data:

The data on chemical parameter and composition in gradients were tabulate and subjected to analysis of variance (ANOVA) as per method to influence of milk yield.

Determining soilds not fat (SNF)% in milk (Prasad *et al.*, 1999):

Principal:

Fat per in milk is determined by Gerber's fat rapidly and total soilds (TS) are determined either by Richmond's or gravitational method. Fat per cent is subtracted from TS and SNF are calculated as follows:

Purpose:

To derminemilk residue without fat which includes mount of proteins lactose and minralmatter, following formula was used:

SNF (%) = T.S.- Fat (%)
where as,
T.S. = Total soilds
S.N. = Soild not fat.

Procedure:

Soild not fat (SNF) was determined by fat from total soild or by Richmod's formula as follows:

Richmods formula:

$$\% \text{ SNF} = \frac{\text{LR at } 15.5^{o}\text{C F}}{45} + 0.14$$

$$= 0.25\ G + 0.20 \times F + F + 0.14$$

where,
LR = Lactometer rea
F = Fat
G = Corrected lactometer reading

RESULTS AND DISCUSSION

The results obtained during the investigation, regarding the chemical qualities of milk have been presented in the preceding chapter, with the help of tables and graphically illustrated. The findings are being discussed in this chapter.

Table 1: Protein (%) in buffalo milk at different milking times

Replication		Milking time			Range		Mean
		M_1	M_2	M_3	Minimum	Maximum	
R_1		5.20	5.10	5.16	5.10	5.20	5.15
R_2		3.65	3.48	3.60	3.48	3.65	3.58
R_3		4.20	4.05	4.15	4.05	4.20	4.13
R_4		3.68	3.60	3.63	3.60	3.68	3.64
R_5		3.40	3.28	3.35	3.28	3.40	3.34
R_6		4.47	4.34	4.43	4.34	4.47	4.41
R_7		3.38	3.15	3.31	3.15	3.38	3.28
R_8		3.70	3.55	3.70	3.55	3.70	3.65
R_9		3.40	3.28	3.35	3.28	3.40	3.34
R_{10}		4.50	4.42	4.46	4.42	4.50	4.46
Range	Minimum	3.38	3.15	3.31			
	Maximum	5.20	5.10	5.16			
	Mean	3.96	3.83	3.91			3.90
					F- test		S
					S. E. ±		0.01
					C. D. (P = 0.05)		0.03

Protein (%) :

Table 1 and Fig. 1 furnish the data on protein (%) in raw buffalo milk at three different milking times in ten replications. The results obtained showed that milking times M_1, M_2 and M_3 registered mean protein (%) as 3.96, 3.83 and 3.91, respectively, with overall mean of 3.90. The difference in these values due to milking times as well as due to replication, was significant, M_1 recorded maximum protein (%)(3.96) followed by M_3 (3.91) while M_2 recorded the minimum (3.83).

Specific gravity (cc):

Table, 2 and Fig. 2 presents the data on specific gravity (cc) of raw milk of buffaloes at three different milking times in ten replications. The results obtained showed that milking time M_1, M_2 and M_3 registered mean

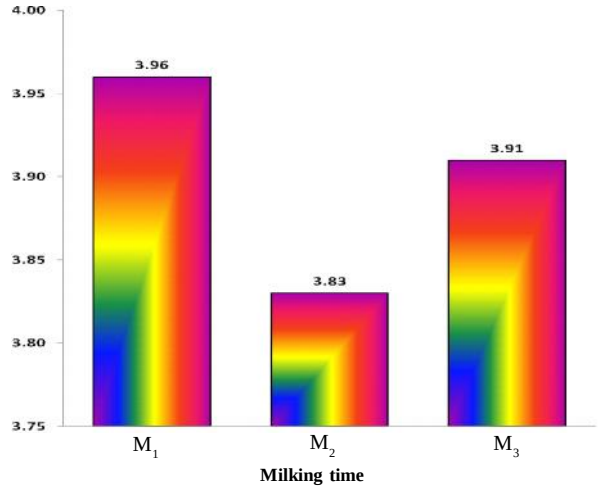

Fig. 1 : Average protein (%) in buffalo milk at different milking times

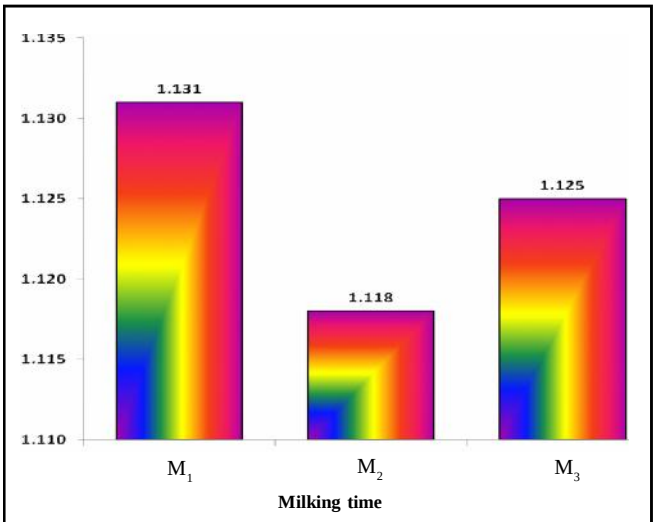

Fig. 2 : Average specific gravity (cc) of buffalo milk at different milking times

Table 2 : Specific gravity (cc) of buffalo milk at different milking times

Replication		Milking time			Range		Mean
		M_1	M_2	M_3	Minimum	Maximum	
R_1		1.139	1.120	1.140	1.120	1.140	1.133
R_2		1.129	1.117	1.124	1.117	1.129	1.123
R_3		1.145	1.136	1.139	1.136	1.145	1.140
R_4		1.130	1.124	1.127	1.124	1.130	1.127
R_5		1.122	1.113	1.118	1.113	1.122	1.118
R_6		1.118	1.103	1.109	1.103	1.118	1.110
R_7		1.141	1.126	1.135	1.126	1.141	1.134
R_8		1.131	1.123	1.126	1.123	1.131	1.127
R_9		1.119	1.103	1.108	1.103	1.119	1.110
R_{10}		1.136	1.115	1.127	1.115	1.136	1.126
Range	Minimum	1.118	1.103	1.108			
	Maximum	1.145	1.136	1.140			
	Mean	1.131	1.118	1.125			1.125
					F- test		S
					S. E. ±		0.001
					C. D. (P = 0.05)		0.003

specific gravity (cc) as 1.131, 1.118 and 1.125, respectively, with overall mean of 1.125. The difference in these values due to milking time as well as due to replication, was significant, M_1 recorded maximum Specific gravity (cc)(1.131) followed by M_3 (1.125), whereas, M_2 recorded the minimum (1.118).

Fat (%):

The data on fat (%) in raw milk of buffalo at three different milking time in ten replications contained in Table, 3 and Fig. 3 showed that milking time M_1, M_2 and M_3 registered mean fat (%)as 5.90, 5.66 and 5.74, respectively, with overall mean of 5.76, The difference in these values due to milking time as well as was due to replication was significant. Morning milk M_1 recorded maximum fat (%) (5.90), followed by evening milk M_3 (5.74), whereas, noon milk M_2 recorded the minimum (5.66).

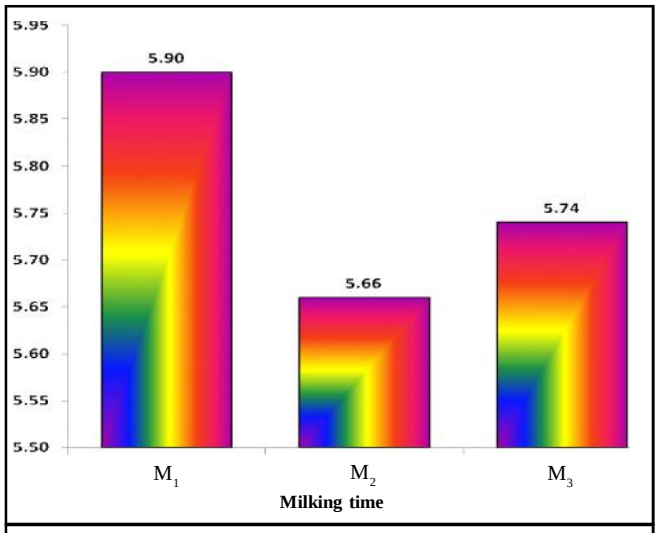

Fig. 3 : Average fat (%) in buffalo milk at different milking times

Lactose (%):

The data on lactose (%) in raw buffalo milk at three different milking times in ten replications contained in Table. 4 and Fig. 4 showed that milking time M_1, M_2 and M_3 registered mean lactose (%)as 4.83, 4.63 and 4.72, respectively, with overall mean of 4.73. The difference in these values due to milking time as well as due to replication was significant. Morning milk M_1 recorded maximum Lactose (%) (4.83) followed by evening milking M_3 (4.72). Noon milk M_2 recorded the minimum (4.63).

Ash (%) :

The data on ash (%)in raw milk of buffalo at three different milking time in ten replications contained in Table. 5 and Fig. 5 showed that morning milk M_1, noon milk M_2 and evening milk M_3 registered mean ash (%) as 0.69, 0.77 and 0.72, respectively, with overall mean of

Table 3 : Fat (%) in buffalo milk at different milking times

Replication		Milking time			Range		Mean
		M_1	M_2	M_3	Minimum	Maximum	
R_1		6.03	5.87	5.95	5.87	6.03	5.95
R_2		5.63	5.39	5.44	5.39	5.63	5.49
R_3		5.58	5.26	5.40	5.26	5.58	5.41
R_4		5.93	5.58	5.74	5.58	5.93	5.75
R_5		6.70	6.45	6.54	6.45	6.70	6.56
R_6		5.68	5.36	5.48	5.36	5.68	5.51
R_7		6.28	6.11	6.20	6.11	6.28	6.20
R_8		5.94	5.71	5.77	5.71	5.94	5.81
R_9		5.95	5.75	5.67	5.67	5.95	5.79
R_{10}		5.27	5.09	5.17	5.09	5.27	5.18
Range	Minimum	5.27	5.09	5.17			
	Maximum	6.70	6.45	6.54			
	Mean	5.90	5.66	5.74			5.76
					F- test		S
					S. E. ±		0.02
					C. D. (P = 0.05)		0.04

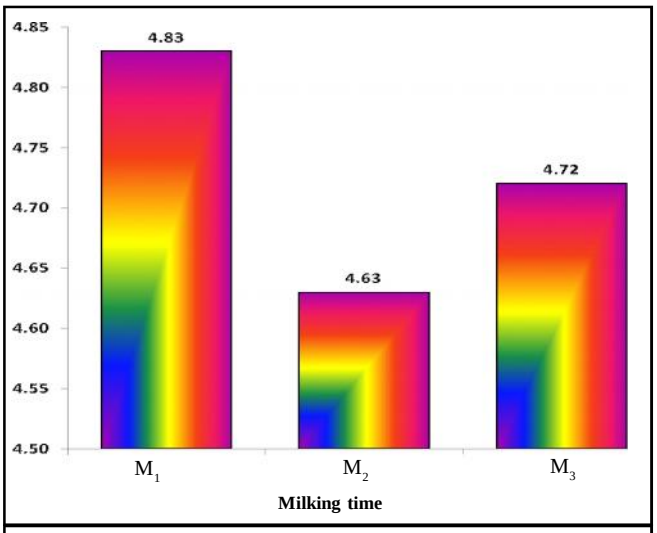

Fig. 4 : Average lactose (%) in buffalo milk at different milking times

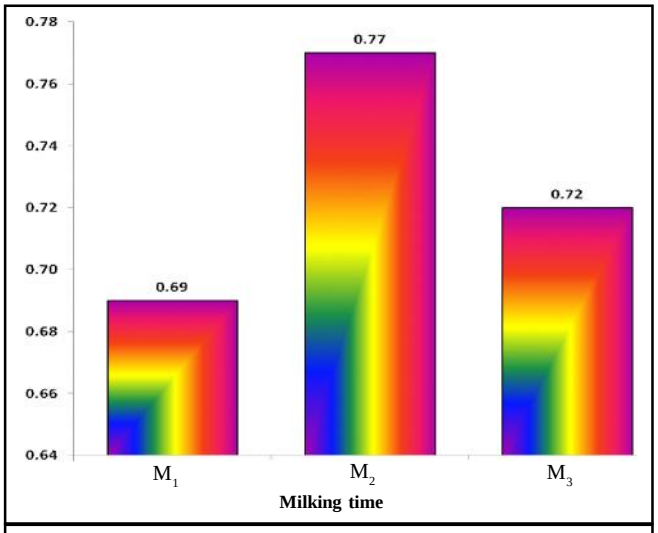

Fig. 5 : Average ash (%) in buffalo milk at different milking times

0.73. The difference in these values due to milking time as well as due to replication were significant. M_1 recorded minimum ash (%)(0.69), whereas, M_2 recorded the maximum (0.77) followed by M_3 (0.72).

Total solid (%):

The data on total solid (%) in raw milk at three different milking time in ten replications contained in Table 6 and Fig. 6 showed that morning milk M_1, noon milk M_2 and evening milk M_3 registered mean total solid (%) as 15.44, 14.68 and 15.13, respectively, with overall mean of 15.09. The difference in these values due to milking time as well as due to replication were significant. Morning milk M_1 recorded maximum total solid (%) (15.44) followed by evening milk M_3 (14.68), whereas, noon milk M_2 recorded the minimum (15.13).

Table 4 : Lactose (%) in buffalo milk at different milking times

Replication		Milking time			Range		Mean
		M_1	M_2	M_3	Minimum	Maximum	
R_1		6.08	6.02	6.06	6.02	6.08	6.05
R_2		4.85	4.63	4.73	4.63	4.85	4.74
R_3		4.47	4.24	4.33	4.24	4.47	4.35
R_4		4.35	4.05	4.20	4.05	4.35	4.20
R_5		4.70	4.47	4.60	4.47	4.70	4.59
R_6		4.17	4.06	4.12	4.06	4.17	4.12
R_7		4.76	4.50	4.64	4.50	4.76	4.63
R_8		6.08	5.92	6.00	5.92	6.08	6.00
R_9		4.48	4.27	4.33	4.27	4.48	4.36
R_{10}		4.35	4.09	4.20	4.09	4.35	4.21
Range	Minimum	4.17	4.05	4.12			
	Maximum	6.08	6.02	6.06			
	Mean	4.83	4.63	4.72			4.73
					F- test		S
					S.E. ±		0.02
					C. D. (P = 0.05)		0.04

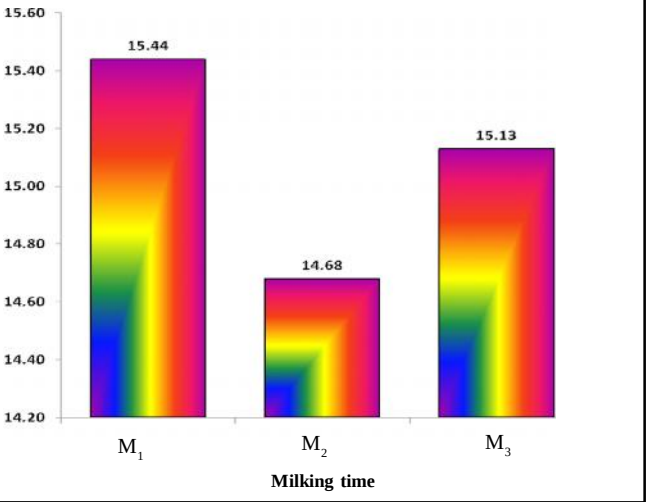

Fig. 6 : Average total solid (%) in buffalo milk at different milking times

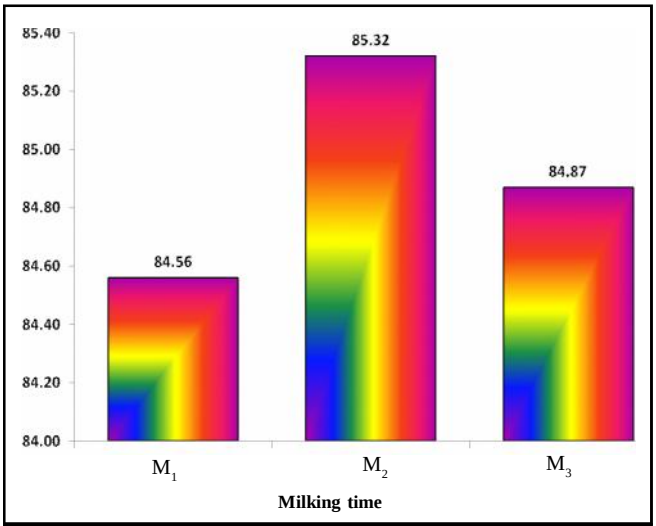

Fig. 7 : Average water (%) in buffalo milk at different milking times

Water (%):

The data on water (%)in raw milk of buffalo at three different milking times in ten replications contained in Table 7. and Fig. 7 showed that milking time M_1, M_2 and M_3 registered mean water (%) as 84.56, 85.32 and 84.87, respectively, with overall mean of 84.92. The difference in these values due to milking time as well as due to replication were significant (Table 7). Morning milk M_1 recorded minimum water (%) (84.56), whereas, noon milk M_2 recorded the maximum (85.32) followed by evening milk M_3 (84.87).

Solid not fat (SNF) (%):

The data on SNF (%)in raw buffalo milk at three different milking time in ten replications contained in Table 8 and Fig. 8 showed that morning milk M_1, noon milk M_2

Table 5 : Ash (%) in buffalo milk at different milking times

Replication		Milking time			Range		Mean
		M_1	M_2	M_3	Minimum	Maximum	
R_1		0.66	0.75	0.71	0.66	0.75	0.71
R_2		0.75	0.84	0.81	0.75	0.84	0.80
R_3		0.61	0.78	0.72	0.61	0.78	0.70
R_4		0.67	0.74	0.70	0.67	0.74	0.70
R_5		0.77	0.84	0.80	0.77	0.84	0.80
R_6		0.70	0.75	0.73	0.70	0.75	0.73
R_7		0.70	0.77	0.71	0.70	0.77	0.73
R_8		0.67	0.76	0.69	0.67	0.76	0.71
R_9		0.64	0.69	0.66	0.64	0.69	0.66
R_{10}		0.68	0.75	0.70	0.68	0.75	0.71
Range	Minimum	0.61	0.69	0.66			
	Maximum	0.77	0.84	0.81			
	Mean	0.69	0.77	0.72			0.73
					F- test		S
					S. E. ±		0.01
					C. D. (P = 0.05)		0.02

Table 6 : Total solid (%) in buffalo milk at different milking times

Replication		Milking time			Range		Mean
		M_1	M_2	M_3	Minimum	Maximum	
R_1		15.93	15.16	15.55	15.16	15.93	15.55
R_2		16.90	15.96	16.48	15.96	16.90	16.45
R_3		14.90	14.18	14.49	14.18	14.90	14.52
R_4		16.76	16.02	16.51	16.02	16.76	16.43
R_5		14.48	13.81	14.15	13.81	14.48	14.15
R_6		15.49	14.92	15.24	14.92	15.49	15.22
R_7		15.25	14.50	15.10	14.50	15.25	14.95
R_8		14.71	13.90	14.32	13.90	14.71	14.31
R_9		15.86	15.12	15.55	15.12	15.86	15.51
R_{10}		14.15	13.26	13.90	13.26	14.15	13.77
Range	Minimum	14.15	13.26	13.90			
	Maximum	16.90	16.02	16.51			
	Mean	15.44	14.68	15.13			15.09
					F- test		S
					S.E. ±		0.03
					C. D. (P = 0.05)		0.07

Table 7 : Water (%) in buffalo milk at different milking times

Replication		Milking time			Range		Mean
		M_1	M_2	M_3	Minimum	Maximum	
R_1		84.07	84.84	84.45	84.07	84.84	84.45
R_2		83.10	84.04	83.52	83.10	84.04	83.55
R_3		85.10	85.82	85.51	85.10	85.82	85.48
R_4		83.24	83.98	83.49	83.24	83.98	83.57
R_5		85.52	86.19	85.85	85.52	86.19	85.85
R_6		84.51	85.08	84.76	84.51	85.08	84.78
R_7		84.75	85.50	84.90	84.75	85.50	85.05
R_8		85.29	86.10	85.68	85.29	86.10	85.69
R_9		84.14	84.88	84.45	84.14	84.88	84.49
R_{10}		85.85	86.74	86.10	85.85	86.74	86.23
Range	Minimum	83.10	83.98	83.49			
	Maximum	85.85	86.74	86.10			
	Mean	84.56	85.32	84.87			84.92
					F- test		S
					S.E. ±		0.03
					C. D. (P = 0.05)		0.07

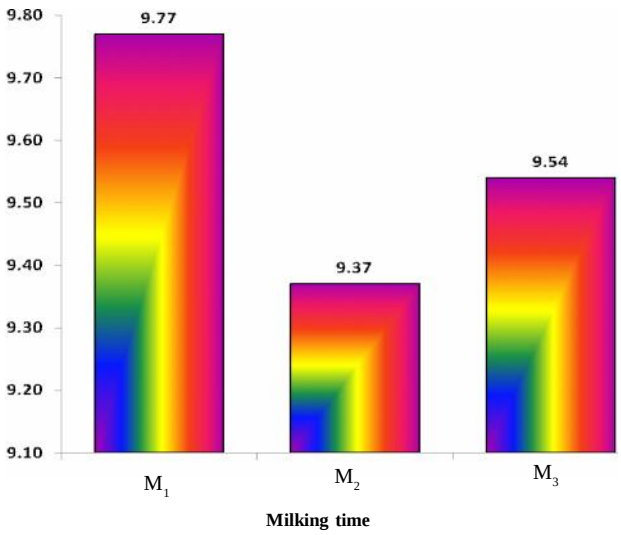

Fig. 8 : Average solid not fat (%) in buffalo milk at different milking times

and evening milk M_3 registered mean SNF (%) as 9.77, 9.37 and 9.54, respectively, with overall mean of 9.56. The difference in these values due to milking time as well as due to replication were significant. Morning milking M_1 recorded maximum SNF (%) (9.77) followed by evening milk M_3 (9.54), whereas, noon milk M_2 recorded the minimum (9.37).

Summary and conclusion:

The data collected for milk of three buffaloes, at morning, noon and evening, for ten days, on different parameters, were subjected to statistical analysis, applying the technique of analysis of variance (F-test).

The results of the investigation regarding the chemical qualities of buffalo milk at different milking times have been presented in tables, graphically illustrated and discussed in the preceding chapters.

Results of the experiment are summarized below:

Maximum protein (%) was recorded in the raw buffalo milk at morning milk M_1followed by evening milking $M_3$and the minimum in noon milking M_2.

Specific gravity (cc) was recorded maximum in the raw milk of buffalo at morning M_1 followed by evening milk M_3 and minimum in noon milk M_2.

Morning milk M_1 recorded the highest fat (%) followed by evening milking M_3 and the lowest fat %) was found in the noon milk M_2.

Maximum lactose (%) was recorded in the morning milk M_1 followed by evening milking M_3 while the minimum remained in noon milk M_2.

Minimum ash (%) was found in the buffalo milk of morning M_1 and the maximum in noon milk M_2 followed by evening milk M_3.

Total solid (%) was recorded maximum in morning

Table 8 : Solid not fat (SNF) (%) in buffalo milk at different milking times

Replication		Milking time			Range		Mean
		M_1	M_2	M_3	Minimum	Maximum	
R_1		10.70	10.60	10.63	10.60	10.70	10.64
R_2		11.38	11.19	11.28	11.19	11.38	11.28
R_3		9.43	9.00	9.18	9.00	9.43	9.20
R_4		8.95	8.54	8.68	8.54	8.95	8.72
R_5		8.95	8.52	8.73	8.52	8.95	8.73
R_6		9.85	9.25	9.46	9.25	9.85	9.52
R_7		10.52	10.15	10.36	10.15	10.52	10.34
R_8		10.48	10.06	10.23	10.06	10.48	10.26
R_9		8.88	8.35	8.56	8.35	8.88	8.60
R_{10}		8.60	8.08	8.29	8.08	8.60	8.32
Range	Minimum	8.60	8.08	8.29			
	Maximum	11.38	11.19	11.28			
	Mean	9.77	9.37	9.54			9.56
					F- test		S
					S.E. ±		0.04
					C. D. (P = 0.05)		0.07

milk M_1 followed by evening milk M_3, whereas noon milk M_2 recorded the minimum.

Water content in the raw milk of buffalo was found in morning milking M_1 while the maximum was recorded in noon milk M_2 followed by evening milk M_3.

Raw milk of buffalo at morning M_1 recorded the highest solid not fat (SNF) (%) followed by evening milk M_3 and the lowest SNF (%) was found in the noon milk M_2.

Chemical quality of raw buffalo milk at morning M_1 was found best, which contained maximum protein (%), specific gravity (cc), fat (%), lactose (%), total solid (%) and solid not fat (SNF) (%) and minimum ash (%) and water (%), followed by evening milk M_3 and noon milkM_2.

In all the parameters, the difference in the mean values due to milking time as well as due to replication was significant.

The order of chemical quality of raw buffalo milk at threes different milking times under study, based on their chemical qualities, was:

$\mathbf{M_1 > M_3 > M_2}$

Conclusion:

In view of the findings and results presented above, it may be concluded that the chemical quality of raw buffalo milk at morning (M_1) was found best in terms of maximum protein (%), specific gravity (cc), fat (%), lactose (%), total solid (%) and solid not fat (SNF) (%); and minimum ash (%) and water (%), followed by evening milk (M_3) and noon milk (M_2).

LITERATURE CITED

Abd El-Salam, M.H. and El-Shibiny, S. (2011). A comprehensive review on the composition and properties of buffalo milk. *Dairy Sci. & Technol.,* **91** : 663–699.

Atherton, H.V. and Newlander, J.A. (1987). *Chemistry and testing of dairy product*', 4th Ed., CBS Publisher and Distributors, New Delhi, India.

Burdova,, Espachs, A., Arantegui, J. and Martin, O. (2002). Effect of high intensity pulsed electric fields and heat treatments on vitamins of milk. *J. Dairy Res.,* **69** : 113-123.

Davies, J.E., Freed, V.H. and Whittemore, F.W. (1986). *An agro medical approach to pesticide management.* Coral Gables, FL: University of Miami School of Medicine.

Dehinenet, G., Mekonen, H. and Ashenafi, M. Emmanuelle (2013). Determinants of raw milk quality under a small holder production system in selected areas of Amhara and Oromia National Regional states, Ethiopia. *Agric. & Biol. J. North America,* **4** (1) : 84-90.

Harding, F. (1999). *Milk quality* (Ist Ed). Chapman and Hall, London, pp.102-104.

Kartheek, M., Smith, A.A., Muthu, A.K. and Manavalan, R. (2011). Determination of adulterants in food: a review. *J. Chem. Pharmacy & Res.,* **3** : 629-636.

Prasad, S., Singh, R. and Bisht, G. S. (1999). Measure of persistency and its relationship with peak yield and lactation milk yield. *Indian J. Dairy Sci.,* **52** : 308-14.

Salle, A. J. (1974). *Fundamentalsprinciples of bacteriology.* Tata McGrawHill Company, Inc. New York,U.S.A.

Sarwar, M., Khan, M.A. and Mahr-Un-Nisa, Z.I. (2002). Dairy Industry in Pakistan: A scenario. *Internat. J. Agric. & Biol.*, **4**: 420 - 428.

Shukla, U.K. and Patel,Vinita (2017). *J. Kalash Science*, **5** (1) : 69-74.

Stewart, T.H. (1978). *An introduction to public health*, Butterworths, Durban.

Yadav, J.S., Grover, S. and Batish, V.K. (1993). *A comprehensive dairy microbiology*, ISBN 81-200-0316-0, Metropolitan Phototype Setters and Printers Ltd, pp. 1-258.

Received : *01.03.2019;* ***Revised:*** *05.04.2019;* ***Accepted :*** *18.05.2019*