Detection of Lactose and Casein in Different Milk Samples

Dadasaheb Navale[1], Shelley Gupta[2]

[1]*Sinhgad Jr. College Vadgaon. Pune.*

[2]*Parvatibai Genba Moze Engineering College Wagholi. Pune*

Abstract: **Milk has been a good source of various nutrients including lactose and casein. We check quality of milk and detection of lactose and casein present in the milk. In this paper various milk samples was analyzed and found that Casein and Lactose values were negligible as compared to its World Health Origin value.**

Keywords**: Polarimeter, Preparation of solution, End point, Standardizations, Nutrition, Fats, different milk samples etc.**

I. INTRODUCTION

Milk is important part of human life. It contains vitamins those play a vital role in milk uses human consumption. Since milk is generally viewed as nutritious food with lots of vitamins, minerals and fats, proteins etc thus used for drinking. It contains vitamins those play prophylactic role in cancer, autoimmune diseases, heart diseases etc .Vitamins play a vital role in milk used for human consumption. Since milk is generally viewed as nutritious food with lots of vitamins, minerals, fats, proteins etc thus used for drinking purpose.

There are different sources of milk samples available, however sufficient information regarding their vitamin present, especially protein, fat etc. Milk is processed into a variety of dairy products such as cream, butter, yogurt, kefir, ice cream, and cheese. Modern industrial processes use milk to produce casein, whey protein, lactose, condensed milk, powdered milk, and many other food-additives and industrial products. This paper detects lactose, casein present in the milk.

II. MATERIALS AND METHODS

For this Buffalo and Cow milk samples were used (each type four samples).All these samples were collected from Anandnagar, Dhyari, Hadapsar, Katraj around Pune in Maharashtra. The samples were kept refrigerated at 4°C and transported to the laboratory within 24 hours, prior to refrigeration. The milk samples were stored at -20°C until analysis.

A. Determination of Lactose in the Milk:

Procedure

Take two graduated flasks, one of 100 ml and the other of 200 ml capacity. Weigh accurately 65·8 g of the prepared sample into each flask. Add to each flask 20 ml of acid mercuric nitrate solution or 30 ml of mercuric iodide solution. To the 1oo ml flask, add phosphotungstic acid solution to the mark, and to the 200 ml, flask, add 15 ml of phosphotungstic acid solution and dilute to the mark with water. Shake both the flasks frequently during 15 minutes, filter through dry filter paper, and polarize. (It is preferable to read solution from 200 ml flask in 400 mm tube to reduce error of reading. Solution from the 100 ml flask may be read in 200 mm tube.)

Calculation:

Calculate percentage of lactose in the sample as follows,

a) Subtract reading of solution from the 200 ml flask (using 400 mm tube) from reading of solution from the 100 ml flask (using 200 mm tube).
b) Multiply difference by 2.
c) Subtract result from reading of solution from the l00 ml flask; and
d) Divide result by 2.

B. Determination of Casein in the Milk

Procedure

Place 10 g of the prepared sample into each of two flasks. Add one milliliter of phenolphthalein indicator solution, followed by 0·4 rnl of the potassium oxalate solution. Set aside for 2 minutes. Neutralize the contents of one of the flasks with the standard sodium hydroxide solution using the other flask as a blank. Add 2 ml of neutralized formaldehyde and again titrate with the standard sodium hydroxide solution to the same pink shade.

Calculation

The first titration value is not required, but the volume in milliliters of the standard sodium hydroxide used in the second titration shall be noted. Multiply this value by 1·38 to obtain the percentage of casein.

III. OBSERVATION TABLE

Sample Description	B_1	B_2	B_3	B_4	C_1	C_2	C_3	C_4	Test Method
Casein %	4.16	4.23	4.08	4.37	3.45	3.17	3.58	3.29	IS 1479 (II) :1961
Lactose %	5.10	5.10	5.20	5.20	4.80	4.70	4.90	4.80	IS 1479 (II) :1961

NOTE:

(1) Buffalo milk samples-B_1, B_2, B_3, B_4 and Cow milk samples-C_1, C_2, C_3, and C_4.

(2) Chemical Analysis was done per 100 gm.

(3) Casein and Lactose values were negligible

IV. RESULTS AND DISCUSSION

The voluminosity of the buffalo milk casein is 2.68-3.72 ml/g at 25-27^{o}C, while that of cow milk casein is 4.18 ml/g. Ahmed (2008) observed that the hydration (solvation) of buffalo casein is lower as compared to casein of cow milk. Kuchroo and Malik (1976) quantified the solvation (hydration) of casein micelles from buffalo milk as 2.60-2.90 g water /g casein compared to 3.48 g water/g casein for cow milk. Ahmad (2008) investigated the modifications of protein-protein and protein-minerals interactions in alkaline pH resulting in miceller disruption in cow and buffalo milk and found that the dissociations took place at pH 9.7 and 8.6 for buffalo and cow milks, respectively. These differences were due to higher concentrations of casein and minerals in buffalo than in cow milk. Animal bioassays have shown the Protein Efficiency Ratio (PER) value of buffalo milk proteins to be 2.74 and that of cow milk as 2.49 (Pandya, 2007).

Milk Proteins: According to Sindhu (1998) buffalo milk has about 11.42% higher protein than cow milk. The concentrations of both, the case in and whey proteins are different **in** cow and buffalo milk. Buffalo milk contains higher caseins and whey protein than cow milk. Whole of the caseins in buffalo milk is present in miceller form while in cow milk only 90-95% is the miceller casein and rest is present in the serum phase. The particle size of the buffalo miceller casein is larger at 110- 160 nm than that of cow miceller casein 70-110 nm (Saraswat, 1985; Commen and Ganguli, 1973).

Macronutrients –Protein Milk is a good source of high quality protein 30. Milk and milk products are the largest source of protein in per school children and the second largest contributor furthr to meat and meat products in all other age groups8,21. Cows‟ milk contains about 3.5% protein by weight, and of this total protein, 80% is casein and 20% whey. Casein is the dominant protein in milk and can be fractionated into four major components: alpha, beta, gamma and kappa-casein. Whey protein is composed predominantly of beta-lactoglobulin and alpha-lactalbumin, but other whey proteins include serum albumin, immunoglobulins (IgA, IgG, IgM), protease peptones, lactoferrin and transferring 30. Carbohydrate the principal carbohydrate found in milk is lactose. Cow‟s milk contains about 4.5g lactose per 100g milk and there is some evidence that lactose is the least cariogenic of the common dietary sugars. In addition, various other components of milk have been considered to be protective against dental caries 31.Fat the fat content of milk varies depending on whether milk is whole, semi skimmed or skimmed.

By virtue of higher opacity of casein micelles coupled with 15 higher levels of colloidal protein, calcium and phosphorous, buffalo milk is more densely white and has superior whitening properties as compared to cow milk (Kuchroo and Malik, 1976).Milk mineral components Milk is an important source of mineral substances, especially calcium, phosphorus, sodium, potassium, chloride, iodine, magnesium, and small amounts of iron. The main mineral com- pounds of milk are calcium and phosphorus, which are substantial for bone growth and the proper development of newborns (Al-Wabel 2008). The high bioavailability of these minerals influences the unique nutritional value of milk.

In a modern European diet, milk is the main source of calcium. Calcium bound to casein (both in organic and mineral form) exhibits significant availability during the milk digestion process (Gueguen and Pointillart 2000); thus, the bioavailability of this element is closely correlated with a higher concentration of casein (Gaucheron 2005).

The highest concentration of this, and other mineral elements, is specific to sheep milk; whereas human and donkey milk contain the smallest amounts of those compounds .Iron concentration in milk is naturally low and influenced by the presence of lactoferrin and xanthine oxidase transferase (Al-Wabel 2008). Iron, zinc, and copper in ruminant milk are related mainly to the casein fraction, whereas in human milk they are connected to soluble proteins (Raynal-Ljutovac and others 2008). Goat milk is characterized by the lowest concentration of iron, zinc, and copper. Camel milk is the richest in these minerals. Despite the low iron concentration in goat milk, iron is more bioavailable in goat milk than it is in cow milk. The explanation for that is that goat milk contains a higher share of nucleotides which contribute to heightened absorption in the intestine (Raynal-Ljutovac and others 2008).

V. PREPARATION OF CASEINS

The casein fraction was treated as described by Olieman et al. The precipitate was washed 3 times with 20 ml of acetate buffer (4% acetic acid and 0.4 M sodium acetate, pH 4.6). In the case of the whole milk sample, an extra wash with 10 ml of dichloromethane was performed to remove lipids, followed by another wash of 10 ml of acetate buffer pH 4.6. The

resulting casein clean fraction was dried, collected, and frozen at -20ºC until use. Casein Sample Buffer (300 mM of Tris, 20 mM of EDTA, 0.1M urea and 10 mM DTT): In a 50 ml flask containing 20 ml of ddi water were dispensed 1.82 g of Trizma base, 0.37 g of EDTA and 0.3 g of Urea, followed by the addition of 0.5 ml of 1 M DTT solution. ddi water was added to a final volume of 50 ml. It may take up to 15 minutes for the complete dissolution of all solids. This solution must be prepared fresh daily.

Preparation of Caseins for CE SDS analysis:300 mg of casein derived from the three varieties of milk was each mixed with 700 µl of the casein sample buffer. The casein fraction generally does not dissolve completely and was therefore left to incubate for 30 min at room temperature. The mixture was centrifuged at 14,000 g for 10 min. The solid was discarded and the supernatant filtered through a 0.45 µm syringe filter. This solution was used for CE SDS analysis.

Preparation of Caseins for CE SDS analysis:50 µl of each filtered sample was thoroughly mixed with 45 µl of CE SDS sample buffer, 2 µl of 10 kD internal standard, and 5 µl of β-mercaptoethanol. This mixture was heated at 100 ºC for 3 minute

REFERENCES

[1]. Department of Health in association with the Welsh Assembly Government, the Scottish Government and the Food Standards Agency in Northern Ireland (2010)The eatwell plate [Online] Available at http://www.nhs.uk/Livewell/Goodfood/Documents/Eatwellplate.pdf
[2]. Children's Health Development Foundation (1998) The Australian Guide to Healthy Eating.[Online] Available at http://www.health.gov.au/internet/healthyactive/publishing.nsf/content/eating/$File/fdpot.pdf
[3]. Chinese Nutrition Society (2007)Dietary Pagoda [Online] Available at www.cnsoc.org
[4]. Food Guide Study Group, The Ministry of Health, Labour and Welfare and the Ministry of Agriculture, Forestry and Fishery (2005) Report of the Food GuideStudy Group: Food Guide Spinning Top [Online] Available at http://www.maff.go.jp/j/balance_guide/b_use/pdf/eng_reinasi.pdf
[5]. US Department of Agriculture Centre for Nutrition Policy and Promotion (2005) Mypyramid. [Online] Available at http://www.mypyramid.gov/downloads/MiniPoster.pdf
[6]. FAO. Food Guidelines by country. Guatemala. Guias Alimentarias para Guatemala (1998) Online ftp://ftp.fao.org/es/esn/nutrition/dietary_guidelines/gtm.pdf
[7]. Drewnowski A et al. (2010) The Nutrient Rich Foods Index helps to identify healthy affordable foods.Am J Clin Nutr 91(Sl):1095S–101S
[8]. Foods Standards Agency (2002) McCance and Widdowson's The Composition of Foods Sixth Summary Edition. Cambridge: Royal Society of Chemistry.
[9]. Marshall TA et al. (2005) Diet Quality in Young Children is influenced by Beverage Consumption J Am Coll Nutr 24(1) 65-75.
[10]. Cadogan J et al.(1997) Milk intake and bone mineral acquisition on adolescent girls: randomised, controlled intervention trial. BMJ 15:315(7118): 1255-60.
[11]. Murphy MM et al. (2008) Drinking flavoured or plain milk is positively associated with nutrient intake and is not associated with adverse effectson weight status in US children and adolescents. J Am Diet Assoc.108(4):631-9.
[12]. Weaver CM (2010) Role of dairy beverages in the diet Physiol Behav.100 (1): 63-66.
[13]. Gregory JR et al. (1995)The National Diet and Nutrition Survey: Children aged 1½ to4½ years.Volume 1: Report of the National Diet and Nutrition Survey. London:HMSO.
[14]. Gregory J et al. 2000)The NationalDiet and Nutrition Survey: Young people aged 4 to 18 years.Volume 1: Report of the National Diet and Nutrition Survey. London:HMSO
[15]. Henderson L et al. (2003)The National Diet and Nutrition Survey: Adults aged 19 to 64 years and over. Volume 1:Types and Quantities of Foods Consumed. London:TSO
[16]. Henderson L et al. (2003) The National Diet and Nutrition Survey: Adults aged 19 to 64 years and over.Volume 2:Energy, Protein, Carbohydrate, Fat and Alcohol Intake.London:TSO
[17]. Henderson L et al. (2003)The National Diet and Nutrition Survey: Adults aged 19 to 64 years and over. Volume 3:Vitami
[18]. J.M.L. Heck,_, C. Olieman, A. Schennink, H.J.F. van Valenberg, M.H.P.W. Visker, R.C.R. Meuldijk, A.C.M. van Hooijdonk, Intl. Dairy Journal, 18(2008)548.
[19]. G. Cartoni, F. Coccioli, R. Jasionowska and M. Masci, J. Chrom. A, 846 (1999)135.
[20]. Ish Kumar Sawhney "Characterizing the engineering properties of skim milk powder and protein derivatives of buffalo milk" Department of Food Engineering and Technology Sant Longowal Institute of Engineering and Technology Longowal – 148106, Punjab February, 2011
[21]. S. Jovanovic, M. Barac, O. Macej, T. Vocic, C. Lacnjevac, Sensors 7(2007)371.
[22]. Butler, L.J. Agricultural and Resource Economics Update3(2)(2000):3-6.
[23]. University of Guelph, Dairy Science Department Website:http://www.uoguelph.ca/foodscience/dairy-science-and technology/dairy-chemistry-and-physics/composition-and-structure-overview
[24]. B. Ribadeau-Dumas and R. Grappin, Review: Milk protein analysis, Lait, 69(1989)35
[25]. Abo El-Nor SAH, Abd El-Gawad MAM, Abd El-Saman MH. 2007. Effect of somatotropin and monensin on the yield and quality of milk and Tallaga cheese from Egyptian buffaloes. Int J Dairy Sci 2(1):58–65.
[26]. Abou Donia MA, Abou-Arab AAK, Enb A, El-Senaity MH, Abd-Rabou NS. 2010. Chemical composition of raw milk and the accumulation of pesticide residues in milk products. Glob Vet 4(1):6–14.
[27]. Abu-Lehia IH. 1987. Composition of camel milk. Milchwissenschaft 42:368–71.
[28]. Abu-Lehia IH, Al-Mohiezea IS, El-Behry M. 1989. Studies on the production of ice cream from camel milk products. Aust J Dairy Tech 44:31–4.
[29]. Ahmad S, Gaucher I, Rousseau F, Beaucher E, Piot M, Grongnet JF, Gaucheron F. 2008. Effects of acidification on physico-chemical characteristics of buffalo milk: a comparison with cow's milk. Food Chem 106(1):11–7.
[30]. Al-Dobaid SN. 2009. Effect of palm oil supplementation on the milk yield and composition of dromedary she camels. Pak J Nutr 8(6):710–4.
[31]. Al Haj OA, Al Kanhal HA. 2010. Compositional, technological and nutritional aspects of dromedary camel milk. Int Dairy J 20(12):811–21.
[32]. Al-Wabel NA. 2008. Mineral contents of milk of cattle, camels, goats and sheep in the central region of Saudi Arabia. Asian J Biochem 3: 373–5.
[33]. Amenu B, Deeth HC. 2007. The impact of milk composition on cheddar cheese manufacture. Aust J Dairy Tech 62:171–84.
[34]. Arsi cA,PrekajskiN, VucicV, TepsicJ,PopovicT,VrvicM ,GlibeticM. 2009. Milk in human nutrition: comparison of fatty acid profiles. Act Vet 59:569–78.
[35]. Aschaffenburg R, Drewry J. 1955. Occurrence of different β-lg in cow's milk. Nature 176:218–9.

[36]. Attaie R, Richter RL. 2000. Size distribution of fat globules in goat milk. J Dairy Sci 83(5):940–4.
[37]. Attia H, Kherouatou N, Dhouib A. 2001. Dromedary milk lactic acid fermentation: microbiological and rheological characteristics. J Ind Microbiol Biotechnol 26(5):263–70.
[38]. Attia H, Kherouatou N, Nasri M, Khorchani T. 2000. Characterization of the dromedary milk casein micelle and study of its changes during acidification. Lait 80(5):503–15.
[39]. Auldist MJ, Kay JK, Thomson NA, Napper AR, Kolver ES. 2002. Concentration of conjugated linoleic acid in milk from cows grazing pasture or fed a Total Mixed Ration for an entire lactation. Proc New Zeal Soc An 62:240–1.
[40]. Auldist MJ, Thomson NA, Mackle TR, Hill JP, Prosser CG. 2000. Effects of pasture allowance on the yield and composition of milk from cows of different β-lactoglobulin phenotypes. J Dairy Sci 83(9):2069–74.
[41]. Bakheit SA, Majid AMA, Nikhala AM. 2008. Camels (Camelus dromedaries)under pastoral systems in North Kordofan Sudan: seasonal and parity effects on milk composition. J Camelid Sci 1:32–6.
[42]. Bansal BK, Hamann J, Lind O, Singh ST, Dhaliwal PS. 2007. Somatic cell count and biochemical components of milk related to udder health in buffaloes. Ital J Anim Sci 6(Suppl 2):1035–8.