Comparative Study of Buffalo and Cow milk Samples Containing Different Vitamins.

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Abstract- **Milk has been a good source of various nutrients including different vitamins. In this paper quality of milk and comparative study of various vitamins present in the milk is done. Various milk samples was analyzed and found that vitamin A has in negligible as compared to its World Health Origin value while vitamins C and D were found absent.**

Keywords-*Vitamins, HPLC, Nutrition, Fats, Milk products and different types of Cow and Buffalo 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. Comparative study between the different types of milk is not available much, so present study was carried out to compare the vitamins present in the milk and to check the quality of 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. All the samples, for vitamin C determination were stabilized with 10% metaphosphoric acid. Upon arrival, the milk samples were stored at -20°C until analysis.

Analytical methods were used for this high performance liquid chromatography (HPLC) as described by Chavez-Servin.

A) Procedure for determination of Vitamin –D & A

Sample Preparation

1) Prepare a 20 IU sample 1gm sample dilute to 10ml
2) Sonicate for 10 minute, Centrifuge it.
3) Inject 20µl upper layer to HPLC
4) For quantitation compare with standard (CRM) of Sigma Aldrich with proper serial dilution Vitamin D/A Final 20 IU

Standard preparation:

1. Weigh 125mg of powder and dilute to 100ml with methanol.
2. Pipette 2ml from above and dilute to 100ml with methanol
3. Again pipette out 2ml from above and dilute to 100ml.
4. The resultant conc. is 20 IU

Calculation

$$\text{Vitamin D} = \frac{\text{Average sample area X Std Wt. x 2 x2 x 40000 x volume i.e. 10}}{\text{Average standard area x 100 x 100 x 100 x sample wt in mg}}$$

Programme

Column Specification: PEERLESS BASIC C18
Dimension: 250 mm X 4.6 mm ID
Mobile Phase: 100 % Methanol
Flow: 1.5ml/min
Column Oven Temp: 30 ^{o}C
Wavelength: 280 nm
Isocratic program: 15 minutes with 100% Methanol

B) Procedure for Vitamin –C

Scope and Reagents-

a) Trichloroacetic acid (TCA) reagent (10%) = dissolve 10g of TCA in 100ml of water.

b) Standard Ascorbic acid solution = Weigh 100mg of L-Ascorbic Acid in 100m V. Flask and dilute to mark with TCA reagent (1000 ppm).

c)Standard Indophenols solution = 50mg of Sodium 2,6-dichlorophenol indophenols stored in desiccators over soda lime in 50ml of water to which 42 mg of Sodium bicarbonate has been added, Then in this add 50mg of sodium dichlorophenol indophenols. Shake vigorously till the indophenols have completely dissolved; dilute it to 200ml with water. Filter the Solution through a filter paper into an amber glass-stopper bottle. Keep the bottle stopper out of direct sunlight and store in a refrigerator.

d) Standardization of Indophenols Solution = Standardize the indophenols solution immediately after it has been prepared.

Transfer 2mL aliquots of the standard Ascorbic Acid solution to 50mL Erlenmeyer flasks containing 5mL of the TCA reagent.

Titrate rapidly with the indophenols solution from a 25mL burette until light but distinct rose-pink color persists for at least 5 seconds.

In the same way, titrate blank composed of 7mL of the TCA reagent along with water. Calculate and express the concentration of the indophenols solution as mg of ascorbic acid equivalent to 1mL of the indophenols solution standardize the indophenols solution daily with freshly prepared standard ascorbic acid solution.

Standard Ascorbic Acid for 1000ppm/1000mg/1000ml

Ascorbic Acid = Indophenol

2mL 26.4mL

2mL is 2mg 26.4mL

i.e 1mL Indophenols= 0.0757mg Ascorbic acid

Procedure-

Preparation of Sample

a) Grind about 5g of sample in a mortar with acid washed sand using TCA reagent then transfer to 100mL volumetric flask. Shake mixture thoroughly then transfer to 100mL with TCA. Filter immediately

b) Take 10mL of the filtrate and titrate rapidly with the Indophenols solution.

c) Carry out a blank determination with 11mL of the reagent along with water sufficient to make the volume of the mixture equivalent to 15mL.

Calculations-

$$\text{Vitamin C} = \frac{A \times B \times 1000 \text{ mg}/100\text{g}}{W}$$

A = Volume of Indophenols for titration

B= Weight in mg of Vitamin C equivalent to 1mL of indophenols solution, W= weight in gm of sample taken.

OBSERVATION TABLE

Sr.No.	Description	Vitamin-A	Vitamin-C	Vitamin-D	Test Method
1	B_1	45 mcg	ND	ND	By HPLC
2	B_2	43 mcg	ND	ND	By HPLC
3	B_3	49 mcg	ND	ND	By HPLC
4	B_4	46 mcg	ND	ND	By HPLC
5	C_1	65 mcg	ND	ND	By HPLC
6	C_2	67 mcg	ND	ND	By HPLC
7	C_3	69 mcg	ND	ND	By HPLC
8	C_4	59 mcg	ND	ND	By HPLC

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

III. RESULTS AND DISCUSSION

Vitamin A (Retinol) was discovered in 1913 when research workers found that certain laboratory animals stopped growing when lard (made from pork fat) was the only form of fat present in their diet, whereas when butter was supplied instead of lard (with the diet remaining otherwise the same) the animals grew and thrived. Further animal experiments showed that egg yolk and cod-liver oil contained the same vital food factor, which was named vitamin A.

The intake recommended by FAO and the World Health Organization (WHO) is 750 µg of retinol per day for adults; lactating mother's need 50 percent more, and children and infants less. It should be noted that these figures are based upon mixed diets containing both vitamin A and carotene. When the diet is entirely of vegetable origin, larger amounts of carotene are suggested, because the conversion from carotene to retinol is not very efficient. The discovery of vitamin C is associated with scurvy, which was first recorded by seafarers who made prolonged journeys.

Opinions regarding human requirements differ widely. It seems clear that as much as 75 mg per day is necessary if the body is to remain fully saturated with vitamin C. However, individuals appear to remain healthy on intakes as low as 10 mg per day. A recommendation of 25 mg for an adult, 30 mg for adolescents, 35 mg during pregnancy

and 45 mg during lactation seems to be a reasonable compromise.

In the nineteenth century, however, scurvy began to occur among infants receiving the newly introduced preserved milk instead of breast milk or fresh cows' milk. The preserved milk contained adequate carbohydrate, fat, protein and minerals, but the heat used in its processing destroyed the vitamin C, so the infants got scurvy. Later vitamin C was found to be ascorbic acid**.**

Ascorbic acid is necessary for the proper formation and maintenance of intercellular material, particularly collagen. In simple terms, it is essential for producing part of the substance that binds cells together, as cement binds bricks together. In a person suffering from ascorbic acid deficiency, the endothelial cells of the capillaries lack normal solidification. They are therefore fragile, and hemorrhages take place. Similarly, the dentine of the teeth and the asteroid tissue of the bone are improperly formed. This cell-binding property also explains the poor scar formation and slow healing of wounds manifest in persons deficient in ascorbic acid.

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