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Milk Constituents and Adulteration Detection

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Abstract: *Milk is considered to be the ideal food or complete food, because of its abundant nutrients. Milk adulterants can cause serious health problems leading to fatal diseases. Now days adequate monitoring required to milk producer farmer from healthy milch animals. This paper presents a review of common milk adulterants as well as different methods to detect the adulterant qualitatively.*

Keywords: Nutritional value of milk, health of consumer, Dairy milk safety, adulteration etc.

I. INTRODUCTION

Milk is a common drink of our daily diet. But not every time the milk we take is pure. The impurities observed due to adulteration. Packed milk from reputed brands as well as milk purchased from vendors can be adulterated. So it's important to check for them before consuming sometime.

History of milk adulteration is very old. The milk scandal has been reported in 1850. Which killed 8000 infants in New York alone. Melamine contamination in Chinese infants milk product, six died and 3000 child ill (1-Feb 2009). Chemical adulteration in milk in Mumbai (9-June 2017, 17-October 2018)[7]

Milk producer farmer adds adulterant in milk for increase milk volume, density and thickness (SNF)[Normally Cow milk contains 8.5% SNF whereas buffalo milk contains 9.0% SNF] they done mixing of buffalo milk in cow milk or cow milk in buffalo milk also. Defrauders adulterated packing milk with dirty water and the artificial milk[6]

II. DISCUSSION

A. Main Constituent of Milk[1,2,3]

1. **Water:** (82.0 to 87.5%): Water is extremely important in human metabolism. Water maintain human body temperature regulation through sweating. Whereas sweating not seen in buffalo and cow.
2. **Fat:** (3.5 to 8.0%): Fat globules varying from 0.1μ to 22μ in diameter. Fat contents in free droplets in emulsion form (true emulsion of oil/butter fat in water), neutral fat, cholesterol and lecithin. The flavor of milk is due to milk fat. Milk fat, is mixture of several different glycerides. They contain 64 different fatty acids ranging from 4 to 26 carbon atoms. Other lipid materials present in milk are phospholipids, sterols, carotenoids and fat-soluble vitamins. The fat is approximately 65% saturated 29% monosaturated and 6% polyunsaturated. The polyunsaturated fatty acid in milk fat include small amounts of the essential fatty acids-linoleic and linolenic acid, that cannot be made by the body and must come the diet and approximately 5% trans fatty acid. An important fatty acid in milk is conjugated linoleic acid.
3. **Protein:** (1.4 to 5.0%): Principal protein casein (Emulsifier, Phospho-protein) lactoglobulin and lactalbumin, casein constitutes 80% of the total nitrogen in milk, whole casein is a heterogenous group of protein, consisting of three principal components α-casein, β-casein, X-casein and Y-casein. 0.5% of nitrogen of 95% protein and 5% non-protein. The non-casein proteins of milk are called whey proteins. These includes β-lactoglobulin, α-lactalbumin, serum albumin and proteos/ peptose fraction. Milk protein consist of approximately 82% casein and 18% whey (serum) proteins. Protein contains leucine, isoleucine, valine, phenylalanine, tryptophan, histidine, threonine, methionine and lysine (nine amino acids) are essential ammino acids are often called complete protein. In most cheeses the casein is coagulated to form the curd. Whey proteins contain immunoglobulins. Whey proteins contain branched chain amino acid-leucine, isoleucine and valine. Milk proteins are rich in lysine
4. **Carbohydrate:** (3.6 to 7.0%): Lactose (4.5 to 5%): Milk sugar is a reducing disaccharide. Which on hydrolysis gives glucose and galactose. Which sugar units both serve as source of energy for cell (for activity also). Lactose

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is acted upon by bacteria to produce lactic acid. Average 250gm of milk provides 100-150 kcal of energy. The fat provides 9 kcal/ gram, Macronutrients protein 4 kcal/ gm and carbohydrates provides 4 kcal/ gm.

5. **Mineral:** (0.2 to 0.9%): Ca, K, phosphate is in large amount Na, Mg and chloride in fair amount, Fe in poor amount. Others Cu, Zn, Mn, Al and iodide in small amount. Milk ash is white residue remaining after incineration of milk at 600^0C. It consists of oxides of Na, K, Ca, Mg, Fe, P and S salts: phosphate, chloride and citrates of Na, K, Ca, Mg.
6. **Vitamins:** Milk is good source of water soluble and fat soluble (A, D, E, K) vitamins. Vitamin K (Coagulation vitamin), vitamin A (β carotene) are present in good quantity in milk. The concentration of niacin (Nicotinic acid), ascorbic acid (vitamin C) is relatively low in milk. Riboflavin (vitamin B_2), Pyridoxine (vitamin B_6) are also present in milk. The vitamin A and E are destroyed in significant amounts in the presence of oxidizing agents. Nearly 50% of thiamine is lost with heating. Riboflavin is lost rapidly on exposure to light.
7. **Enzymes:** More than 25 enzyme have been identified in cow's milk. Milk contains alkaline phosphates, amylase, lipase, xanthine oxidase, galactase and reductase etc.

Composition of milk from various species[2] (Constituents in gram / 100 cm^3)

Sr.No	Species	Water	Fat	Protein	Lactose	Ash/ Minerals
1.	Buffalo	82.14	7.44	4.78	4.81	0.83
2.	Cow	87.27	3.66-4.00	3.47-3.5	3.66-4.8	0.69-0.7
3.	Goat	84.14	6.0	4.03	6.0	5.02
4.	Human	87.47	3.50-3.76	1.4-2.14	3.76-7.0	0.31-0.2

All solids in the milk are referred to as total solids (11.4-14.5%) and the total solids without fat known as milk-solids-no-fat (MSNF) or solids-not-fat.

PFA standards for different classes and designations of milk in India.

Class of milk	Milk fat	MSNF
Cow Milk	4.0	8.5
Mixed Milk	4.5	8.5
Standardized Milk	4.5	8.5
Recombined Milk	3.0	3.76
Toned Milk	3.0	8.5
Doubled Tones Milk	1.5	9.0
Skimmed Milk	Not more than 0.5	8.7
Full Cream Milk	6.0	9.0

Types of milk: Lactose free milk, Fat free milk(or Skimmed Milk), Organic milk.

Milk density value: Buffalo's milk 1.032 to 1.034. Cow's milk 1.028-1.029. Specific gravity (Weight of milk/ml) of whole milk is usually around 1.30. The specific gravity of milk is determined by lactometer. (specific gravity range is 1.025 to 1.035 at 28.8º (OR 84º F)

B. Detection: Detection of milk adulteration[4,5]

Qualitative and quantitative methods for detection of adulteration in milk were known. Qualitative detection of adulterant in milk can be easily performed with chemical reactions. While quantitative detection is complex and diverse. Milk is adulterated with adulterant to increase thickness of the milk. Starch and urea are used to increase the specific gravity of milk, so that lactometer fails to detect the adulteration of milk with water.

Type of quantitative detection techniques depend on the nature of adulterants in milk e.g. liquid chromatography (LC), Enzyme linked immunosorbent assay are the most common techniques used to detect foreign protein, polymerase chain

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reaction (PCR) and polyacrylamide gel electrophoresis (PAGE) are usually used to detect milk from different species as adulterants in milk of a particular species.

Table-1: Rapid qualitative detection of different edible adulterants in milk.

Sr. No	Adulterant	Procedure	Observation
1.	Sugar[2,6] (cane sugar)	Take 5ml to 10ml milk sample in test tube. Add 1ml to 5ml conc.HCI and 0.1gm resorcinol solution, shake the tube content well. Place the test tube in water bath for 5 min.(Warm)	Appearance of red colour indicates the presence of added sugar.
2.	Starch[2,6]	Take 3ml milk sample in test tube after boiling it thoroughly, cool it to room temperature. Add 1 to 3 drops 1% iodine solution.	Appearance of blue colour indicates the presence of starch.
3.	Glucose	Take 1ml to 3ml of milk sample in test tube. Add 1 to 3ml Barford's regents. Keep the test tube in boiling water bath for 3min then cool it for 2min under the tap water without disturbance. Add 1ml of phosphomolybdic acid reagent to turbid solution and shake.	Immediate appearance of deep blue colour indicates the presence of glucose.
4.	Water	Putting a drop of milk on a polished slanting surface. Let it flow down lactometer reading detects adulteration of milk with water.	The drop of pure milk flows slowly leaving a white trail behind it. Milk adulterated with water will flow immediately without leaving a mark.
5.	Common salt	Take 1ml to 5ml of milk sample in a test tube. Add 1 to 5ml of 0.1N $AgNO_3$ solution. Mix the content thoroughly and add 0.5ml (or 2 to 3 drops) of 10% potassium chromate solution.	Appearance of yellow colour indicates the presence of added salts, whereas brick red colour (chocolate or reddish brown in colour) indicates the milk free from added salts.
6.	Skim milk powder	Take 5ml milk sample in test tube. Add drop by drop nitric acid	Development of orange colour it indicates the milk is adulterated with skim milk powder sample with-out skim milk powder shows yellow colour.
7.	Vanaspati/ Dalda in milk	Take 5ml milk sample in test tube 2 to 5ml HCI and add 1 to 2 gm sugar	If mixture turns red. The milk is impure.

Table-2: Rapid qualitative detection of different hazardous chemicals in milk.

Sr. No.	Adulterant	Procedure	Observation
1.	Urea[6]	Take 5ml milk sample in test tube. Add 2gm of soyabean powder (arhar). Mix up the contents thoroughly by shaking the test tubes. After 5 minutes dip a red litmus paper after half a minute.	A change in colour from red to blue indicates the presence of urea in milk.
		Take 5ml milk sample in test tube. Add 1ml of 2% NaOH and boil the content.	Smell of ammonia indicates sample is adulterated.
		Take 5ml milk sample in test tube. Add 1ml of conc. HCl and 1ml of 1% $NaNO_2$.Shake the content.	Bubbles of nitrogen arise from the sample. The sample is adulterated.
		Take 5ml milk sample in test tube. Add 1ml 2%NaOH followed by 1ml of 2% sodium hypochlorite solution and 1ml of 5% phenol solution. Shake the content.	Blue or bluish green colour develops indicates sample is adulterated (The colour is stable for 12 hrs)

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		Take 5ml milk sample in test tube. add 0.2ml of urease. Shake well and then add 0.1ml of Bromothymol Blue (BTB) solution (0.5%).	The appearance of blue colour after 15 minutes indicates the presence of added urea.[2]
		Take 5ml milk sample in test tube. Add 5ml of p-dimethyl amino benzaldehyde reagent.	The appearance of distinct yellow colour indicates the presence of added urea. Whereas the appearance of slight yellow colour indicates the presence of natural urea.[2]
		15ml milk sample, add 1 gm sodaash well.	pH greater than 7.5, sample adulterated[6].
2.	Hydrogen Peroxide[2]	Take 1ml milk sample in test tube. Add 1ml potassium iodide-Starch solution reagent and mix well.	Appearance of blue colour indicates the presence of H_2O_2 as adulterant.
3.	Formalin[2]	Take 10ml milk sample in test tube. Add 5ml conc.H_2SO_4 along the side of wall.	Appearance of violet or blue colour at the junction of two liquid layers indicates the presence of formalin.
4.	Ammonium Sulphate[2]	Take 2ml milk sample in a test tube. Add 0.5ml NaOH (2%) 0.5ml sodium hypochlorite (2%) and 0.5ml phenol (5%). Heat in boiling water bath for 20 second.	A bluish colour forms immediately. Which turns deep blue afterward. Pure milk shows salmon pink colour which gradually changes to bluish after 2 hours.
		Take 5ml hot milk in a test tube. Add suitable acid eg. Citric acid and whey obtained is separated and filtered. Take whey in another test tube and add 0.5ml of 5% Barium Chloride.	Appearance of precipitate indicates the presence of ($NH_4 SO_4$)
5.	Benzoic acid and Salicylic acid[2]	Take 5ml of milk in a test tube. Add 3-4 drop of conc.H_2SO_4. Add 0.5% $FeCl_3$ solution drop by drop and mix well.	Development of buff colour indicates presence of benzoic acid and violet colour indicates presence of salicylic acid.
6.	Borax and Boric acid[2]	Take 5ml milk sample in a test tube. Add 10ml conc. HCl acid and mix well. Dip the tip of turmeric paper into the acidified milk and dry in a watch glass at 100^0 C (or over a small flame) Add a drop of ammonium solution on the turmeric paper.	If the turmeric paper turns red, it indicates the presence of borax or boric acid. If the red colour changes to green it confirms the presence of boric acid.
7	Nitrate[2]	Take 10 ml of milk in beaker, add 10ml of mercuric chloride solution and mix well. Filter the solution through Whatman filter paper number 42. Take 1.0ml this filtrate in test tube, add 4.0 ml of Diphenyl amine sulphate or diphenyl reagent.	The appearance of blue colour indicates the presence of nitrate.

Table-3: Rapid qualitive detection of different mixed adulterants in milk

Sr. No.	Adulterant	Procedure	Observation
1.	Detergent[2]	Shake vigorously 5 to10ml of milk sample with an equal amount of water.	Formation of lather (foam or bubbles persist longer than usual) indicates the presence of detergent

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		Take 5ml of milk sample in a test tube and add 0.1ml 0.5% Bromocresol purple (BCP)solution. Mix well.	Appearance of violet colour indicates the presence of detergent. Unadulterated milk shows faint violet colour.
		Take 5ml of milk sample into 15ml test tube. Add 1ml of methylene blue dye solution and 2ml chloroform. Vortex the contents for about 15 sec and centrifuge at about 1100 rpm for 3 minutes.	Relatively more intense blue colour in lower layer indicates presence of detergent in milk. Relatively more intense blue colour in upper layer indicates absence of detergent in milk.
2.	Pulverized soap[2]	Take 10ml milk sample in a test tube. Add equal quantity of hot water to it. Then add 1 to 2 drop of phenolphthalein indicator.	Development of pink colour indicates that the milk is adulterant with soap.
3.	Colouring matter[2]	Take 10ml milk sample in test tube. Add a few drops of HCl to milk sample.	Appearance of pink colour indicates azo dyes.
		Take 10ml milk sample in test tube. Make the milk sample alkaline with bicarbonate. Dip a strip of filter paper for 2 hours.	Appearance of red colour on filter paper indicates the presence of annatto treatment of this paper with stannous chloride gives pink colour.
		Take 10ml milk sample in test tube. Add 10ml diethyl ether. After shaking allow it to stand.	Appearance of yellow colour in ethereal layer indicates the presence of added colour.
4.	Synthetic Milk	Take 5ml synthetic milk in test tube. It give a soapy feeling on rubbing between the fingers. Heat it.	It turns yellowish.

Other tests for adulterated milk:

1. Addition of turmeric powder in milk sample develops red colour.
2. Addition of ladies' finger powder in milk sample gives foam appearance.
3. Tea made with milk sample (if $NaHCO_3$ in milk) then tea appears pink colour.
4. Simply by taking taste of milk sample, the sugar, sodium chloride, water etc. adulterants detected.
5. Addition of water in milk can be detected by cryoscopy instrument method.

Detection of Neutralizers in Milk[5]

Prohibited neutralizers like hydrated lime, NaOH, Na_2CO_3 or $NaHCO_3$[6] are added to milk to prevent spoilage.[7]

Rosolic acid test (soda test)

Take 5ml of milk sample in a test tube and add 5ml ethyl alcohol (70%) followed by 2 to 3 drops of rosolic acid (1%). if the colour of milk changes to pinkish red. It is inferred that the milk is adulterated with Na_2CO_3 or $NaHCO_3$ and so unfit for human consumption.

Alkaline condition of the milk for the presence of soda ash. Alkalinity test:

Take 20ml of milk in a silica crucible and evaporate the water. The contents are then burnt in a muffle furnace at 550^0 C. The ash is dispersed in 10ml distilled water and titrated against 0.1N HCl acid using phenolphthalein indicator. If the titre value exceeds 1.2ml it can be construed that the milk is adulterated with neutralizers.

Detection of buffalo milk in cow milk:

Tested by Hansa test.

It is based on immunological assay.

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1ml of milk is diluted with 4ml of water. It is then treated with 1ml of antiserum. The characteristic precipitation reaction indicates the presence of buffalo milk in the sample take. (antiserum is developed by injecting buffalo milk proteins into rabbits)

Detection of vegetables fat in milk:

The characteristic feature of milk is in its fatty acid composition, which mainly consist of short chain fatty acids such as butyric, caproic, caprylic acid. Whereas the vegetable fats consists mainly of long chain fatty acids and hence adulteration of vegetable fat in milk can be easily found out by analysing the fatty acid profile by gas chromatography.

II. CONCLUSION

Increasing population all over the world, inadequate supply of milk to customer, demand and supply of gap, perishable nature of milk and lack of suitable detection test, milk adulteration is acute problem developing in India. It is observed (10 Oct 2019,News)that in India, packet milk has not 38% standard value. Thus, it is necessary to dairy milk company should testing the milk in laboratory of food safety and standards authority of India and receive quality certificate and then sold milk in local market[7]

Defrauders doing milk adulteration for financial gain. For milk adulteration detection specific and rapid techniques are needed. Milk adulteration is hazardous or injurious to health, that must be prevented.

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