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Forensic Detection and Identification of Some Milk Adulterants

Vinod Dhingra[1], AK Jaiswal[2]

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Abstract

Food forensics is an emerging branch of Forensic Chemistry which comes to rescue when food is contaminated/ adulterated. It helps to check food safety and quality amongst others. Nowadays, food adulteration is a common problem and different countries are at stake due to lack of monitoring and infringement of the policies. However, adulteration in milk is one of the most common problems that have been overlooked in many countries. Unfortunately, milk adulterants can pose serious health problem leading to fatal diseases. This study basicallyfocuses on common milk adulterantswhich are widely used to adulterate milk and other dairy products using different methods, which are employed to detect the adulterants both qualitatively and quantitatively. this paper deals with chemicals which are used for adulterating milk and the techniques used for their detection which is usefulfor awareness among Forensic Chemist and Toxicologist.

Keywords: Milk Processing; Food Adulteration; Milk Adulterants etc.

Introduction

Food forensics is an emerging branch of forensic chemistry which comes to rescue when food is contaminated/adulterated. It helps to check food safety and quality amongst others. Milk is an emulsion or colloid of butterfat globules within a water-based fluid that contains dissolved carbohydrates and protein aggregates with minerals. Because it is produced as a food source for the young, all of its contents provide benefits for growth. The principal requirements are energy (lipids, lactose, and protein), biosynthesis of non-essential amino acids supplied by proteins (essential amino acids and amino groups), essential fatty acids, vitamins and inorganic elements, and water.

Milk is the single most important food material of human diet. Milk is one of the best sources for protein, vitamin and minerals etc. It is considered to be the complete food for humans' beings. Adulterant means any material which is unsafe or sub-standard or misbranded or containing extraneous matter. Milk is considered to be adulterated, if its quality is lowered or affected by the addition of substances which are injurious to health.[1-3]

Adulterants in milk mainly with vegetable protein, milk from different species, addition of whey and watering which are known as economically motivated adulteration. These adulterations do not pose any severe health risk. The adulterants such as urea, formalin, detergents, ammonium sulphate, boric acid, caustic soda, benzoic acid, salicylic acid, hydrogen peroxide, sugars and melamine etc have severe health risk which can be stopped at any cost. Ureabeing a natural constituent of raw milk has a maximum limit fixed by Food Safety and Standards Authority of India (FSSAI) Act 2006 and

Author's Affiliation: : [1]Senior Scientific Officer, Regional Forensic Science Laboratory, Gwalior, Madhya Pradesh, [2]Chemist, Department of Forensic Medicine and Toxicology, All India Institute of Medical Sciences, New Delhi.
Correspondence: Vinod Dhingra, Senior Scientific Officer, Regional Forensic Science Laboratory, Gwalior, Madhya Pradesh
E-mail: vdhingraso@hotmail.com

Prevention of Food Adulteration Rules 1955 which is to be 70 mg/100 ml. Different adulterants with their purposes and maximum limit fixed by FSSAI is given in table 1. Since, milk fat is very expensive, some manufacturers of milk and dairy products remove milk fat for additional financial gain and compensate it by adding non-milk fat such as vegetable oil. Detergents are added to emulsify and dissolve the oil in water giving a frothy solution, which are the desired characteristics of milk.[4,5]

S.No	Adultrants	Purposes for adding
1	Urea	To increase non-protein nitrogen content and improve the whiteness of milk
2	Melamine	To increase protein content falsely
3	Ammonium sulphate	To increase the lactometer reading by maintaining the density of diluted milk
4	Salicylic acid	act as preservatives and increase the shelf life of the milk
5	Benzoic acid	Act as preservatives and increase the shelf life of the milk
6	Hydrogen peroxide	Act as preservatives and increase the shelf life of the milk
7	Vegetable oil	Compensate milk fat
8	Detergents	Emulsify and dissolve the oil in water giving a frothy solution, which are the desired characteristics of milk

Adulterated milk and its effect on health

The adulteration of milk is banned due to the harmfull effects. The effects of the various adulterants used can vary from high to relatively mild, such as reducing the nutritional value of milk, to potentially fatal, like cancer. For example, milk adulterated with water would naturally be less nutritious than unadulterated milk. The side effects of ingesting urea include indigestion, diarrhoea, acidity, malfunctioning of kidneys, damage to the intestinal tract and digestive system, ulcers and impaired vision. Formalin is a solution made up of water and formaldehyde. Formaldehyde, is chemical widely used to embalm human and animal remains. Formalin is added to prolong the shelf life of milk. Side effects of drinking milk adulterated with formalin include mood and balance alteration, liver and kidney damage, and abdominal pain, among others. Carbonate in milk produce gastrointestinal problems including gastric ulcer, diarrhoea, colon ulcer and electrolytes disturbance. Chloride in the milk disturbs the acid base balance in the body and also blood pH. Ammonia in milk develops regression, loss of acquired speech and sensory disturbances. Possible side effects of consuming milk laced with detergents include gastro-intestinal complications, hypotension, respiratory irritation and cancers. However, urea overburdens the kidneys as they have to filter out more urea content from the body.

Qualitative Methods for Detection And Identification of Some Common Adulterants in Milk

Qualitative detection of adulterants in milk can be easily performed with chemical reactions. Different tests are performed for different adulterants. Common milk adulterants and their qualitative tests are as follow

Sugar

1. 5 ml of milk sample is taken in a test tube.
2. 1 ml HCl and 0.1g resorcinol is added to it.
3. Test tube is kept in water bath for 3-5 minutes.
4. Appearance of red color indicates the presence of added sugar.

Starch

1. 3 ml milk sample in a taken in a test tube boil and boiled
2. Boiled milk is cooled at room temperature.
3. 1 drop of 1% iodine solution is added to it.
4. Appearance of blue color indicates the presence of starch

Glucose

1. 1-2 ml of milk sample is taken in a test tube.
2. 1ml of modified Barfoed's reagent is added to it.
3. Test tube is kept over boiling water bath for 2-4 minutes
4. Test tube is cooled under the tap water.
5. Iml of phosphomolybdic acid reagent is added to the above test tube
6. Immediate appearance of deep blue color indicates the presence of glucose.

Common salt

1. 5 ml of milk sample is taken into a test tube.

2. 1ml of 0.1N silver nitrate (AgNO3) solution is added to it.
3. 0.5ml of 10% potassium chromate solution is added to it.
4. Appearance of yellow color indicates the presence of common salts, whereas, brick red color indicates that milk is free from added salt.

Buffalo milk

1. Dilute the milk by 1/10 in a test tube.
2. Put a drop of diluted milk on the centre of a glass slide.
3. Now place a drop of Hansa test serum on the drop of milk and mix together with a glass rod or clean tooth pick.
4. Curdy particles develop within half a minute in the presence of buffalo milk.

Hydrogen peroxide

Method 1.

1. 5ml of suspected milk sample is taken in a test tube.
2. An equal volume of raw milk and 5 drops of 2% solution of para-phenylenediamine is added to it.
3. Appearance of blue color indicates the presence of hydrogen peroxide as adulterant.

Method 2

1. 1 to 2 ml milk sample is taken in a test tube
2. 1ml of potassium iodide-starch reagent solution is added to it and mixed well.
3. Appearance of blue color indicates the presence of hydrogen peroxide as adulterant.

Formalin

Method 1

1. 10ml milk sample is taken in a test tube.
2. 5ml conc. sulfuric acid is added with a little amount of ferric chloride without shaking.
3. Appearance of violet or blue color at the junction of two liquid layers indicates the presence of formalin.

Method 2

1. 5ml of milk is taken in a test tube.
2. Take 1ml of 10% ferric chloride solution in a 500ml volumetric flask and make up the volume using concentrated hydrochloric acid. Add Sml from this solution to the sample in test tube.
3. Test tube is kept on boiling water bath for about 3-4 min.
4. Appearance of brownish pink color indicates the presence of formalin.

Method 3

1. 1-2 ml of sample milk is taken in a test tube.
2. Saturated solution of 1, 8-dihydroxynaphthalene-3, 6- disulphonic acid is made in about 72% sulfuric acid to make chromotropic acid solution.
3. 1 ml of chromotropic acid solution is addedinto test tube.
4. Appearance of brownish pink color indicates the presence of formalin.

Ammonium sulfate

Method 1

1. 2 ml milk is taken in a test tube.
2. 0.5 ml, 2%NaOH , 0.5 ml 2% sodium hypochlorite and 0.5ml 5% phenol are added to it.
3. Heat the content in boiling water bath for 20 -30 sec.
4. A bluish color forms immediately, which turns deep blue afterward. Pure milk shows salmon pink color which gradually changes to bluish after 2hours.

Method 2

1. 10 ml of milk sample is taken in a 50ml stoppered test tube.
2. 10 ml of TCA solution is added to it.
3. Filter the coagulated milk through Whatman filter paper Grade 42.
4. 5 ml of clear filtrate is taken in a test tube.
5. Few drops of barium chloride solutionis added to it.
6. Formation of milky-white precipitates indicates the presence of added sulfates like ammonium sulfate, sodium sulfate, zinc sulfate and magnesium sulfate etc.

Urea

Method 1

1. 5 ml milk sample is taken in a test tub.

2. Equal volume of 24% TCA is added to it to precipitate fat and proteins of milk.
3. Filtered the solution and 1ml filtrate is taken in another test tube
4. 0.5 ml 2% sodium hypochlorite, 0.5 ml 2% sodium hydroxide and add 0.5 ml 5% phenol solution are added and mixed.
5. A characteristic blue or bluish green color develops in presence of added urea whereas pure milk remains colorless.

Method 2

1. 5 ml milk is taken in a test tube.
2. 0.2 ml urease (20mg/ml) is added to it and shaken well at room temperature.
3. 0.5%, 0.1ml Bromothymol Blue (BTB) solution is added to it.
4. Appearance of blue color after 10-15 minutes indicates the presence of urea in milk. Normal milk shows faint blue color due to natural urea present in milk.

Method 3

1. 5 ml milk sample is taken in a test tube.
2. 5 ml p-Dimethylaminobenzaldehyde reagent is added to it.
3. Appearance of distinct yellow color indicates presence of added urea whereas formation of slight yellow color indicates natural urea in milk.

Nitrate

1. 10 m1 sample milk is taken in a beaker.
2. 10 ml mercuric chloride solution is added to it.
3. After mixing, filtered through what man No. 42 filter paper.
4. 1 ml filtrate is taken in a test tube and 4ml of diphenyl amine sulphate or diphenyl benzidine reagent are added.
5. Appearance of blue color indicates the presence of nitrates. Pure milk sample will not develop any color.

Benzoic and salicylic acid

1. 5 ml milk sample is taken in a test tube.
2. 2.5 drops of sulfuric acid 0.5% ferric chloride solution is added to it drop by drop and mixed well.
3. Development of buff color indicates presence of benzoic acid and violet color indicates salicylic acid.

Borax and boric acid

1. 5 ml milk sample is taken in a test tube.
2. l ml conc. HCI is added to it.
3. A turmeric paper is dipped in test tube
4. Paper is taken out and dried in a watch glass at 100°C.
5. Turmeric paper turns redindicates the presence of borax or boric acid.

Detergent

Method 1

1. 5 ml milk sample is taken in a test tube.
2. 0.1ml 0.5% Bromocresol Purple (BCP) solution is added to it.
3. Appearance of violet color indicates the presence of detergent. Unadulterated milk shows faint violet color.

Method 2

1. 5 ml of milk sample is taken into a 15 ml test tube.
2. 1 ml of Methylene blue dye solution and 2ml chloroform is added to it.
3. Vortex the contents for about 15sec and centrifuged at about 1100 rpm for 3-4 min.
4. Relatively, more intense blue color in lower layer indicates presence of detergent in milk.
5. Relatively more intense blue color in upper layer indicates absence of detergent in milk.

Pulverized soap

1. 10 m1 milk sample is taken in a test tube.
2. Equal quantity of hot water and 2 drops of phenolphthalein indicator is added to it.
3. Appearance of pink color indicates presence of soap.

Coloring matter

Method 1

1. 10 ml milk sample is taken in a test tube.
2. 10 ml diethyl ether is added to it.
3. Test tube is shaken well and allowed it to stand.

4. Appearance of yellow color in ether layer indicates the presence of added color.

Method 2

1. 1-2 ml milk sample is taken in a test tube.
2. Few drops of hydrochloric acidis added to it.
3. Appearance of pink color indicates azo dyes.

Quantitativemethods for Detection and Identification of Some Common Adulterants in Milk

Type of quantitative detection techniques depend on the nature of adulterants in milk. For example, LC (Liquid Chromatography) and ELISA (Enzyme Linked Immunosorbent Assay) are the most common techniques used to detect foreign protein; PCR (Polymerase Chain Reaction) and PAGE (Polyacrylamide Gel Electrophoresis) are usually used to detect milk from different species as adulterants in milk of a particular species. Milk adulteration detection techniques need to be very specific and rapid, because defrauders have escaped condemnation claiming less effectiveness of the conventional detection techniques.

Different Technique employed for quantitative estimation common adulterants are

1. *Soy milk*: Polarimetric method, Isoelectric precipitation, SDS-PAGE (Sodium = Dodecyl Sulfate Polyacrylamide Gel Electrophoresis), HPLC (High Performance Liquid Chromatography) and immune-diffusion method.
2. *Vegetable protein*: NIR (Near Infra Red) spectroscopy
3. *Soy, pea, and wheat proteins*: ELISA
4. *Soy, pea, brown rice and hydrolyzed*: Ultra High Performance Liquid wheat protein Chromatography).
5. *Cow milk adulteration in caprine*: HPLC/ ESI-MS (High Performance Liquid milk Chromatography/Electrospray Ionization-Mass Spectroscopy).
6. *Bovine, ovine and caprine milk*: RP-HPLC (Reverse Phase High Performance mixtures Liquid Chromatography)
7. *Cow milk adulteration in goat, sheep*: ELISA and buffalo milk
8. *Cow milk in ewe milk, goat cheese*: PCR and in buffalo mozzarella cheese
9. *Melamine*: SERS (Surface Enhanced Raman Spectroscopy) SB-ATR FTIR (Single Bounce Attenuated Total Reflectance-Fourier transform infrared spectroscopy) Laser Raman spectroscopy
10. *Melamine in both liquid and powder*: SB-ATR FTIR (Single Bounce Attenuated Total milk Reflectance-Fourier transform infrared spectroscopy)
11. *Urea*: Near infrared Raman _ spectroscopy, LC, GC/IDMS (Gas Chromatography/ Isotope Dilution Mass Spectrometry), HPLC. An enzyme based pizo-electric sensor, EISCAP (Electrolyte Insulator Semiconductor Capacitor), a potentiometric biosensor based on enzymatic reaction
12. *Melamine, urea and ammonium*: A combination of kjeldahl and sulphate spectrophotometric method
13. *Water and whey in cow milk*: NIR spectroscopy (1100-2500 nm)
14. *Synthetic urea*: Infrared microspectroscopy and chemometric analyzes flame atomic absorption spectroscopy
15. *Vegetable oil in milk*: MALDI-QTOF MS_ (Matrix-assisted Laser Desorption / Ionization Time of Flight Mass Spectroscopy)
16. *Ammonium sulfate*: Raman chemical imaging melamine and urea in milk powder
17. *Milk fat adulteration Butyro*: Refractome, flucencespectroscopy,derivative spectroscopy and Raman spectroscopy
18. *Antibiotics*: Electrical conductivity BRT Test (Test kit) Spot Test Penzyme milk test, SNAP test and LACTEK test (kits) Chromatography (HPLC) Liquid chromatography mass _ spectrometry Somatic cell count (SCC) Screening test Biosensor assay based on surface plasmon resonance (SPR) E-Nose
19. *Color*: Capillary electrophoresis
20. *Chlorine*: Sequential Injection Analyzes (SIA) Flow Injection Analyzes (FIA) Potentiometric detection Conducto metric sequential injection analyzes
21. *Neutralizers*: Conductivity or pH measurement

Conclusion

Forensic Chemistry uses the several principles of chemical techniques to aid investigation agencies and law enforcement. Food Forensics is a field

of study under the forensic chemistry division and deals with the identification and analyzes of illegalities in relation to food products or items of local consumption. Milk is a vulnerable target for economically motivated adulteration. One of the main reasons for milk adulteration is the financial procurement but inadequate supply of milk for such a large population all over the world has created the path for this. The problem is more severe in developing and under developed countries due to incapable law enforcement agencies. The combined efforts of scientific communities and law regulating bodies will leads to the development and implementation of better tools and techniques for the estimation of adulterants in milk. Along with this, awareness among the individual towards their health hazards can play a pivot role in controlling this hazardous problem. Some of these easy detection methods at the consumer level and instrumental techniques at the authority level can bring this problem to an end for the victims, including millions of children in the developing countries.

The adulterant decreases the nutritive value of milk/milk products and may also cause serious human health problems. Food adulteration may lead to chronic poisoning, various kinds of diseases and even fatality. Forensic studies with regards to food have not been a widely popular especially in India, where most of the cases of adulteration and testing are routinely carried out by the Food and Drug Administration. The study is carried out keeping in view the recently emerging concern of adulteration of natural milk with various illegal substances to increase its marketability. This article creates awareness among us towards some commonly used adulterants and their simple detection techniques to get the power to make right choice to select their healthy food. This study is an effort to create awareness among forensic chemist/scientist and toxicologist for detection and identification of different adulterants in milk.

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