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A Review

Review on adulteration of milk and milk products and detection

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The problem of detection of adulteration in *Ghee* is very complex inspite of having the constant values for *Ghee*, because as it has already been seen that these constant values are always subject to changes even due to natural factors.

If R.M. value is above 28, and Polenske value is also correct, *Ghee* may be taken as genuine. When R.M. value is between 24 and 28, other tests should also be made and if the value is below 24, the genuineness of the sample is very doubtful. However, due to wide variation in these constant values, care must be taken in the judgement of purity of the *Ghee*. Moreover, there is no test which may distinguish cow's *Ghee* though adulterated to the extent of 20 per cent may pass through as genuine cow's *Ghee* if R.M. value and P. value, saponification value and refraction readings are relied upon.

Valenta test:

Three ml. of the suspected sample is mixed with 3 ml. of glacial acetic acid, in a test tube, which is dipped in warm water. Agitate frequently and note the temperature at which it melts. If it melts between 29ºC and 39ºC, then the sample is genuine but if melting point is above 39ºC, it indicates the addition of some foreign animal fat. An abnormally low figure suggests coconut oil.

Another detection test for foreign animal fat is to dissolve one part of suspected sample in 2:25 pars of dilute carbolic acid (9:1:acid:water). Allow to stand, Foreign animal fat rises to the top, while butter fat or *Ghee* is dissolved by the acid.

Halphens test for cotton seed oil:

Five ml of amyl alcohol and 5 ml. of 1 per cent solution of sulphur in carbon di-sulphide is mixed with 10 ml of suspected sample. This is heated on an oil bath at a temperature of 120º-130 º C for one hour. If the sample contains even 1 per cent cotton seed oil a red colour appears.

Nitric acid test:

This test detects the presence of vegetable *Ghee*. The basis of this test is that the nitro compounds of fatty

acids and their esters formed by the action of nitric acid on pure *Ghee* are colourless, while those with vegetable *Ghee*, tallow or wax are coloured.

Three ml. of the suspected sample are melted by heating in water. Two or three drops of pure and colourless nitric acid is acid is added to it and it is again kept in hot water. In case *Ghee* is unadulterated no colour appears otherwise the colour changes to yellow, orange or reddish brown, depending upon the nature and amount of impurity. The vegetable fat produces deep yellow, tallow or lard orange, wax-reddish yellow colour. When mixed colour appears, it indicates the presence of a mixture of the above.

Soda ash test:

This test is based upon the fact that pure *Ghee* is very slightly saponified by Na_2CO_3, while *Ghee* or oil, tallow or wax is readily saponified.

The suspected sample is melted and an equal amount of 25 per cent solution of Na_2CO_3, is added. It is shaken and kept in boiling water. In case *Ghee* is pure, the liquid becomes turbid due to fat globules and no soap is formed, and on standing two separate layers, one of melted *Ghee* above the other layer of soda ash solution are formed. But if *Ghee* is impure soap is formed by the combination of Na_2CO_3, and vegetable oil, tallow or lard. On standing the soap separates out in form of white or yellow layer, the lower layer consists of excess Na_2CO_3. The amount of soap formed depends on quantity of impurity.

Recent trends in detection of adulteration of milk and dairy products:

Detection of adulterants in milk:

Simple and rapid methods have been developed to detect various adulterants in milk. The ingredients of synthetic milk are also detected by specific tests for urea, ammonium sulphate, detergents, vegetable oils etc.

Some of these tests are discussed below:

Detection of removal of fat by skimming:

The following indicates this:

– Lower percentage of fat
– Higher density reading
– Higher ratio of SNF; fat

Detection of added waster:

The following indicates this:

– Lower percentage of fat
– Lower percentage of SNF
– Lower density reading
– Depression of freezing point.

Water is the most common adulteration and its presence can be detected by testing the freezing point of milk the AOAC specifies a freezing point for normal milk of -0.55°C and the percentage of added water is calculated as follows:

$$\text{Percentage of added water} = \frac{0.550\text{-}T \times 100}{0.550}$$

T is the freezing point depression (FPD) of suspected milk sample. FPD of pure milk is 0.550.

Detection of starch:

Starch, cereal flours or arrowroot are added to make up the density of milk to prevent detection of added water. It is detected by starch-iodide test.

Three ml well mixed sample is taken in a test tube. It is heated to boil over flame, cooled to room temp. A drop of 1 per cent iodine solution is added and mixed. Appearance of blue colour indicates the presence of starch which disappears on boiling and reapperas on cooling.

Detection of cane sugar:

It is added to raise the density to prevent detection of extraneous water. To about 10 ml milk in a test tube, add 1 ml conc. HCL and 0.1 g resorcinol and mix place the test tube in boiling water bath for 5 min, In the presence of cane sugar (sucrose), red colour is produced.

Detection of glucose:

Whereas the test for detection of cane sugar is simple, that of glucose is not so. For this reason, glucose may be added to milk instead of sucrose.

Take 1 ml milk protein – free filtrate and add 1 ml, modified Barfoed reagent. Heat in boiling water batch for 3 min and cool under tap water for 2 min then add 1ml phosphomolybdic acid reagent and mix. Development of deep blue colour indicates the presence of glucose in milk, Pure milk shows faint bluish due to diluted Barfoed reagent.

Detection of sodium chloride:

Sodium chloride (common salt) is added to make up the density (lactometer reading) of watered milk.

Take 2 ml of milk and add 0.1 ml of 5 per cent

potassium chromate and 2 ml of 0.1 N silver nitrate. Appearance of yellow precipitate indicates the presence of sodium chloride.

Detection of ammonium sulphate:

Like urea, ammonium sulphate is a chemical fertilizer, which is added to milk to raise the density of watered milk.

Take 2 ml. milk in a test tube and add 0.5 ml NaOH (2%) 0.5 ml socium hypochlorite (2%) and 0.5 ml phenol (5%) Heat in boiling water bath for 20 sec. A bluish colour forms immediately, which turns dep blue afterward.

Pure milk shows salmon pink colour which gradually changes to bluish after 2 hours.

Detection of urea:

Like ammonium sulphate, urea is a chemical fertilizer, which is added to watered milk to make up its density (lactometer reading) Being an important ingredient of synthetic milk, it is also used in milk to raise its SNF content several methods have been developed to detect adulteration of milk with added urea. It is noteworthy that urea is also a natural constituent of milk. The average content of urea in cow milk is about 50 mg/100 ml whereas in buffalo milk it is present to the extent of 35 mg/100 ml (average). It is also important to note that feeding of urea as a protein supplement in the ration of dairy animals does not help to increase the urea content of milk substantially. However, concerned investigations need to be taken up in this direction as the menace of urea adulteration in milk is rising day by day.

Test (i):

Take 5 ml, milk and add equal volume of 24 per cent trichloroacetic acid (TCA) to precipitate fat and proteins of milk. Filter and collect filtrate take 1 ml. filtrate and add 0.5 ml. sodium hypochlorite (2%), 0.5 ml. sodium hydroxide (2%) and 0.5 ml phenol solution (5%) and mix.

Test (ii):

Take 5 ml milk in a test tube, add 0.2 ml urease (20 mg/ml) Shake well at room temperature and then add 0.1 ml Bromothymol blue (BTB) solution (0.5%). Apopearance of blue colour after 10-15 min. indicates the presence of urea in milk. Normal milk shows faint blue colour due to natural urea present in milk.

Test (iii):

Take 5 ml milk in a test tube and add 5 ml of p – dimethyl amino benzaldehyde (DMAB) reagent (1.6% in ethyl alcohol containing 10% HCI) development of distinct yellow colour denotes the presence of added urea. The pure milk sample shows a slight yellow colour due to the presence of natural urea in milk.

Processing treatments such as chilling, pasteurization and boiling of milk as well as adulterants and neutralizers do not affect the determination of added urea in milk (Bector *et al*., 1998).

The test is more sensitive when it is conducted on protein free filtrate obtained as in case of test (i).

Detection of detergent in milk:

Take 5 ml in a test tube and add 0.1 ml Bromocresol purple (BCP) solution (0.5%) appearance of violet colour indicates the presence of detergent in milk pure normal milk shows only faint violet colour.

Detection of pulverized soap:

It is also an ingredient of synthetic milk like detergents. Soaps are defined as sodium or potassium salts of fatty acids. Hence, to detect the presence of pulverized soaps, iodine value, refractive index, fatty acid composition, salt ratio etc. are excellent methods. The presence can also be detected by qualitative method as follows:

To 10 ml. of milk in a test tube, 10 ml. hot water is added followed by 2-3 drops of phenolphthalein indicator. Development of red/pink colour denotes the presence of soap in milk.

Detection of synthetic milk:

Neutralisers such as caustic soda, caustic potash sodium carbonate, sodium bicarbonate and lime water etc. are commonly added to milk to neutralize the developed acidity in milk. Some of these chemicals (neutralizers) are also ingredients of detergents which are major components of synthetic milk. the neutralizers added to milk are detected as follows:

Test (i):

To above 5 ml milk in a test tube, add 5 ml of alcohol and a few drops of rosolic acid (1 % alcoholic solution) and mix well. Appearance of rose red colour indicates the presence of sodium carbonate or bicarbonate neutralizer in milk, Pure milk shows only a brownish

colouration.

Test (iii) alkalinity of ash:

Neutralisation of milk with lime, soda ash or caustic soda increases the ash content, and total alkalinity of the ash from a fixed quantity of milk.This is detected by ashing accurately measured 20 ml of milk and titrating the ash after dispersing in 10 ml water. If the amount of standard 0.1 N hydrochloric acid required to neutalise the alkalinity exceeds 1.20 ml, it indicates the presence of neutralisers in milk.

Detection of colouring matter:

It is a common practice to adulterate buffalo milk with water and sell it as cow milk after adding some yellow colour to it. The following colours are generally used:

– Artificial colours
– Coaltar dyes
– Annatto
– Turmeric
– Some of these dyes are permitted only in some dairy products but none in milk. These are detected as follows:

Test (i):

To 10 ml milk in a test tube, add 10 ml diethyl ether and shake vigorously. Allow to stand. Presence of any colour is indicated by yellow colour of the ethereal layer.

Test (ii):

Add sodium bicarbonate to milk to make it alkaline. Immerse a strip of filter paper for 2 hours. Red yellow colour observed on filter paper indicates the presence of annatto. Treatment of paper with stannous chloride turns pink.

Test (iii):

Add a few drops of hydrochloric acid to milk. Development of pink colour indicates azo (coaltar) dyes.

Detection of buffalo milk added to cow milk:

Where there is a great demand for cow milk the buffalo milk is generally diluted with water and mixed with cow milk to meet the shortages in demand. It is easily detected by Hansa test for this test Hansa test serum is required.

First dilute the milk 1/10. put a drop of diluted milk on the centre of a glass slide. Now place a drops of Hansa test serum (duly preserved) on the drop of milk and mix together with a glass rod or clean tooth pick. Curdy particles develop within half a minute in milk containing buffalo milk.

Detection of formaldehyde:

Formalin (40 % aqueous solution of formaldehyde) is the most common preservative added to milk. The addition of any kind of preservative to milk is legally prohibited. Yet, market samples of milk are occasionally found adulterated with formaldehyde or hydrogen peroxide. Formalin (formaldehyde) added to milk is detected by Hehner test as folllow:

To about 10 ml milk is a test tube. About 5 ml concentrated sulphuric acid containing traces of ferric chloride is added slowly along the side of the test tube so that it forms a layer at the bottom, without mixing with the milk. The development of a violet or blue colour ring at the junction of the two liquids indicates the presence of formaldehyde the test may be combined with the determination of fat nothing whether a violet colour forms on addition of sulphuric acid in the butyro meter.

Detection of hydrogen peroxide:

This is another preservative which is frequently used in milk to prolong its keeping quality.

Add to about 5 ml of milk (suspected sample) in a test tube, an equal volume of raw milk and 5 drops of a 2 per cent solution of paraphenylene diamine. A blue colour is developed in presence of hydrogen peroxide.

Note: Hydrogen peroxide in destroyed when milk is heated or stored for a long period.

Detection of nitrates (Pond water) in milk:

Sodium and potassium nitrates are oxidizing agents and hence, act as preservative pond water also contains appreciable quantities of nitrates and such water is usually admixed with milk by rural milk producers or vendors.

– Take 10 ml milk in a beaker and add 10 ml mercuric chloride solution (2.5% in 1% HCI) mix well and filter through what man No.42 filter paper.

– Take 1 ml filtrate in a test tube and add 4 ml of diphenyl amine sulphate or diphenyl benzidine reagent development of blue colour indicates the presence of nitrtes.

Table 1: Recent trends in detection of adulteration

Ghee may contain BHA not more than 0.02% as antioxidant

Table 4: Agmark standards of *Ghee*

Sr. No.	Tests	All India	Winter regional	Summer
1.	B audouin	Negative	Negative	Negative
2.	Phytosterol acetate	Negative	Negative	Negative
3.	B.R. reading (40°C)	40.0-43.0	41.5-44.0	42.5-45.0
4.	R.M.value (Minimum)	28	23.0	21.0
5.	Polenske value	1.0-2.0	0.5-1.2	0.5-1.0
6.	Moisture (%)	Maximum	0.3	
7.	Free fatty acids (as % Olic acid)			
	Special grade (Red label)	Not more than	1.4	----------
	General grade (Green label)	Not more than	2.5	
	Standard grade (Chocolate label)	Not more than	3.0	----------
	Where cotton seed is exclusively fed to milch animal			

Detection of vegetable fat:

In synthetic milk milk fat is replaced by vegetable fat or oil (refined oil) Thus, vegetable fat/oil is the chief source of fat in synthetic milk. When synthetic milk is admixed with cow or buffalo milk, the presence of Vegetable oil/fat becomes evident, which can be easily detected by one or more of the following methods:

Detection by measuring analytical constants:

The adulteration of vegetable fat in milk can be detected by etracting the fat either by rose. Gottieb method or fat extracted in butyrometer (special butyrometer having both end open) and measuring its physico- 'chemical characteristics such as Butyro refractometer (BR) reading, Relichert – Meissi and Potenske values.

Baudouin test:

Hydrogenated vegetable oil (vanaspati) is a common adulterant in milk fat. Its presence in milk fat can be detected by the fact that sesame oil (minimum 5%) is added in vanaspati by the law. Thus, the presence of this oil in milk fat indicates the presence of vanaspati or sesame oil.

To 5 ml melted milk fat in a test tube, add 5 ml conc, HCI and 0.4 ml furfural solution (2% distilled not earlier than 24 hr. in alcohol). Shake vigorously for 2 minutes and allow the mixture to separate. The development of red or pink colour in acid layer indicates the presence of sesame oil, which is confirmed by adding 5ml water and shaking again. If colour in acid layer persists, sesame oil/vanaspati is present.

Analytical characteristics of adulterant oils and fats:

It is necessary to understand the physico chemical characteristics of adulterant oils and fats to assess the nature and extent of changes in these characteristics of *Ghee* as a consequence of adulteration. Table 5 describes some of these analytical characteristics.

Methylene blue reduction (MBR) test for cotton seed oil:

This test is based on the cylopropenoic fatty acids present in cotton seed oil which quickly reduce the methylene blue dye. Reduction of methylene blue denotes either the presence of cotton seed oil in milk fat or *Ghee* from cotton tract area. Normal *Ghee* does not reduce methylene blue. Similar work related to the present investigation was also carried out by Arora *et al.* (2004); Dubey and Gupta (1986); Ghodekar *et al.* (1974); Kumar *et al*. (2002); Kumar *et al*. (1998); Panda and Bindal (1998a and b); Sharma and Gupta (1982); Varadaraj and Nambudripad (1982) and Varadaraj *et al.* (1983).

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